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BULLETIN

OF THE NEW YORK ACADEMY OF MEDICINE

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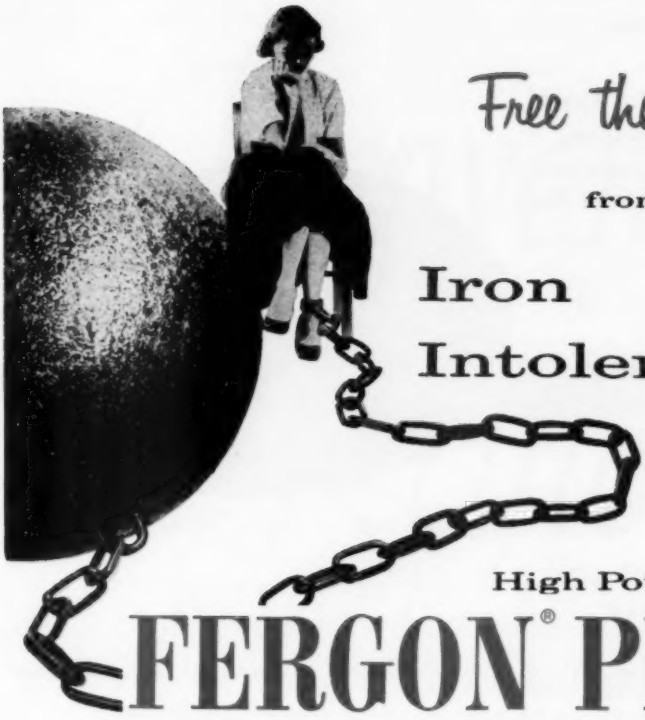

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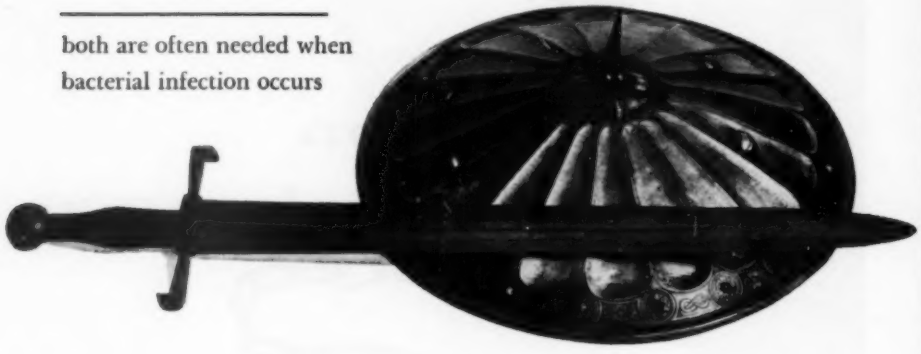

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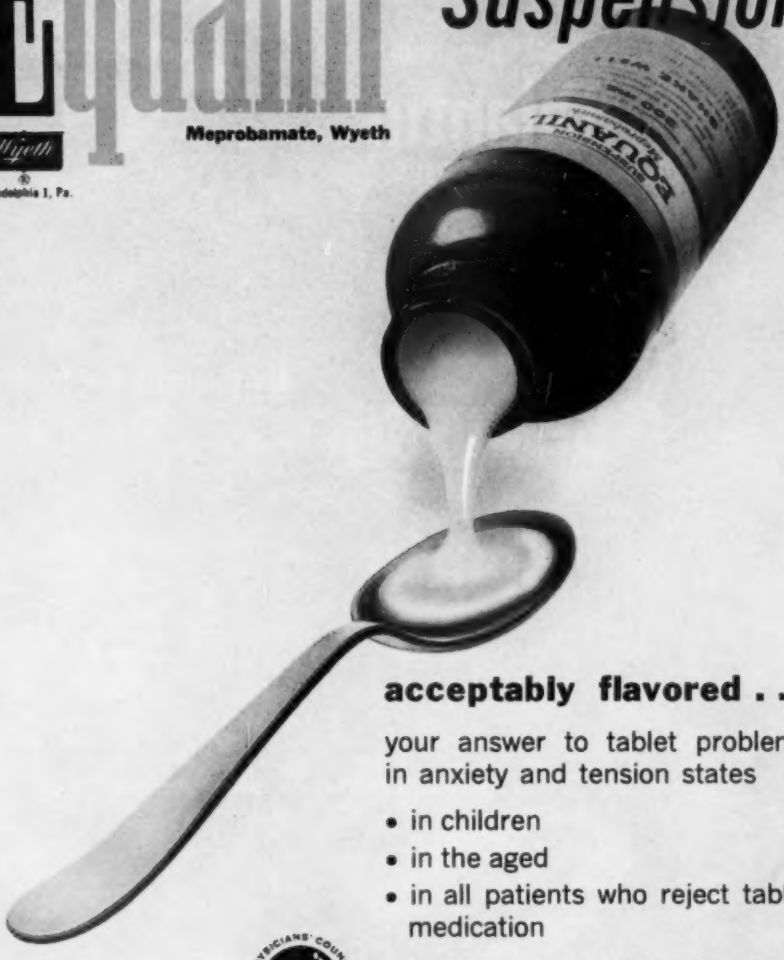

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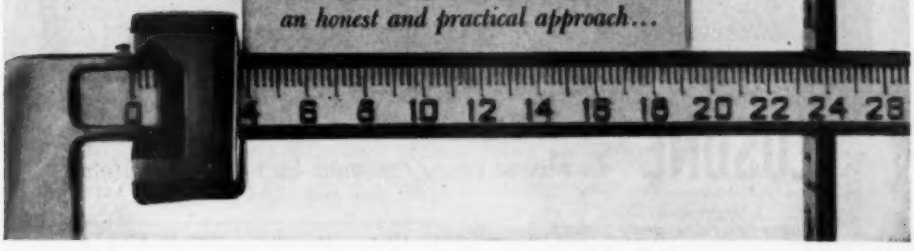

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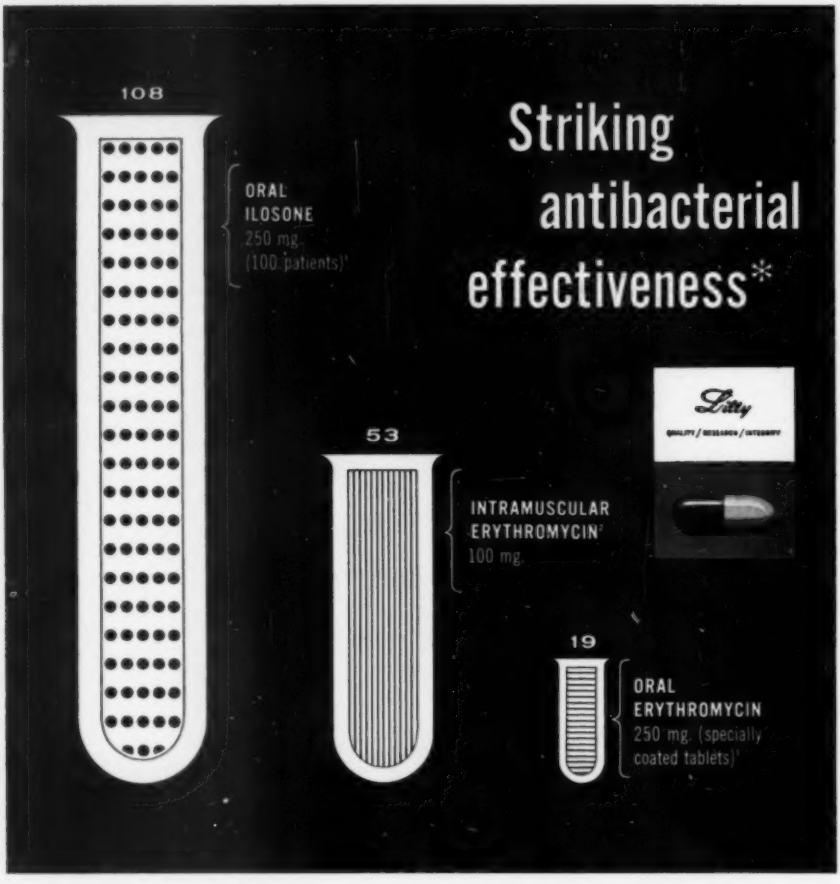

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AUTHORS ALONE ARE RESPONSIBLE FOR OPINIONS EXPRESSED IN THEIR CONTRIBUTIONS

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BULLETIN OF
THE NEW YORK ACADEMY
OF MEDICINE

VOL. 35, NO. 1 JANUARY 1959

ENVIRONMENT AND DISORDERS OF THE CIRCULATORY SYSTEM*

The Second Bernard Sutro Oppenheimer Lecture

GUNNAR BIÖRCK†

Associate Professor of Clinical Medicine, University of Lund; Head of the Division of Cardiology, Department of Medicine, Malmö General Hospital, Malmö, Sweden

It is a great honor and a great privilege for me to address this audience. It is a privilege because I personally owe so much to American medicine; moreover it is a special privilege to give a lecture which is named after Dr. Bernard Sutro Oppenheimer.

Dr. Oppenheimer, in the early years of this century, already had personal contacts with leading European cardiologists, such as Einthoven and Sir Thomas Lewis. During half a century, Dr. Oppenheimer was active as a medical explorer and a teacher, and his fine qualities and distinguished and noble personality are familiar to many of you. His investigations have covered several areas of physiology and clinical medicine, with cardiovascular diseases being his primary interest. In this field he has increased our knowledge about hypertensive encephalo-

* Presented at the Stated Meeting of The New York Academy of Medicine, April 3, 1958.
† As of May 1, 1958, Clinical Professor of Medicine, The Caroline Institute; Head of the Department of Medicine, Serafimerlasarettet, Stockholm, Sweden.

pathy, electrocardiographic changes in acute myocardial infarction, neurocirculatory asthenia, and the effect of tobacco on the peripheral circulation, to name only a few topics basic to the subject that is to be discussed this evening. While some research workers must specialize in one instrumental technique, there is an equally great need of those who manage to retain a holistic attitude to medicine. The complexity of the processes, by which man is modelled in health and disease has always been a challenge to men like Dr. Oppenheimer.

The subject which I have been asked to discuss this evening–*Environment and Disorders of the Circulatory System*–is indeed a broad one, and one which I have accepted with hesitation. Nevertheless, in attempting to deal with it I want to pay tribute to Dr. Oppenheimer for his pioneering work in the study of cardiovascular diseases.

Introduction

Most people who get sick try to project the cause of the disease to something outside their "self", either backwards, to their heredity, or to environmental influences of their surroundings. With regard to heart disease, there have been great efforts to locate the cause of such illness in man's environment. This is not difficult with regard to heart diseases of infectious origin, such as rheumatic and syphilitic heart disease, or some chronic pulmonary heart disease. In other conditions, such as congenital heart disease, coronary heart disease and hypertensive heart disease, the scientific evidence regarding the influence of environmental factors is still quite controversial, both in respect to many of the data presented and to their proper interpretation.

There are several reasons for this. One is the inherent difficulty in studies of causal relationships in diseases, where the "period of incubation" is not known, but is probably long and also variable. Another one may be found in the emotional resistance to far-reaching changes in the mode of living, which appear to be the practical conclusion of some recent studies. An urgent demand from the lay public for information and advice has brought out prematurely to the public some scientific debate and given rise to many over-simplified statements, some of which seem to acquire the status of acknowledged truth.

It has appeared to me, that it might be worth while to examine what we as physicians can accept today as reasonable evidence of environmental influences in disorders of the circulatory system. Our

concept of etiology and of contributing factors in the mechanism of disease forms the basis of our therapeutic activity. To the extent that environment—in its widest sense—can be blamed for disease, it ought to be "corrected"—individually or by general means. Or, perhaps better, the interaction between environment and individuals could be modified. Before entering upon a discussion of the various environmental factors, I would like to consider first the *modus operandi*, the mechanisms by means of which "environment" can influence our circulatory system.

How does "environment" affect us? Primitive organisms are almost entirely dependent upon their immediate environment; the ameba is carried by the stream and surrounded by its food supply. It must accept its environment, for good or evil. Higher up the scale, animals have found ways to cheat a heartless environment: birds migrate to the lands of sunshine, leaving behind them hedgehogs and marmots to withdraw into hibernation. Man alone has found ways to create a new environment for himself, and is doing it rapidly. It is in this man-made —and perhaps biologically harmful—environment that some disorders of the circulation appear to become an ever increasing problem.

Since the time of Claude Bernard we have been taught that the constancy of our internal environment, *le milieu interieur*, is the secret by which we enjoy our external liberty. However true this statement may be, it must not deceive us. Homeostasis is an elastic system, ready to absorb and neutralize moderate and instantaneous impacts from the external environment, but it affords no lasting protection against iterated or chronic trauma, and health may be ruined by "stress diseases".

Many of us are inclined to consider environmental factors as affecting ourselves from the outside, somewhat at a distance. However, they do not affect us unless they become incorporated in us. One part of our environment we inhale, another we absorb through the gastrointestinal system. Climate affects us by its demands upon our own thermostat and the conscious concept of our environment and what is happening in it derives from the elaboration of our sense data by the central nervous system. The immediate relationship between environment and ourselves can be modified *outside* ourselves by various protective devices, or *within* ourselves, as in the alienation from the outer world by some psychotics or by the "tranquilizing" effect of some drugs. Environment, therefore, seems to have two connotations: the "real" one and the one that actually affects us. If we shall get to know about the

"effects" of environment on our circulatory system, we cannot restrict ourselves to empirical or statistical generalities; we must know *how* external environment does creep in under our skin and interferes with the delicate equilibrium of our internal environment—physical and psychological.

Perhaps one of the problems raised by the subject of our discussion is: *If* heart disease is partly due to environment, will it then be easier to do something about it by changing the external environment than by influencing, by various means, including drugs, education and other mental conditioning, the internal one? Shall we attempt to change that which we incorporate, or shall we attempt to modify our equipment for assimilation and disposal of the outer world? Preventive medicine aims at the former target, biochemistry and neurophysiology at the latter.

"Host" and "agent". In the Biggs Lecture on the *Epidemiology of Heart Disease*, in this Academy a year ago, Paul D. White[1] made some remarks about "the *host*, ranking at least as high as, if not indeed higher than [that of] the *agent*" in the causation of heart disease. Using slightly different terms, I would contrast the genetic equipment and the accumulated environmental influences as "causes" of heart disease (with the possible addition that intrinsic cellular decay may be a third factor in old age, which is less specific and perhaps universal).

Some of the difficulties in the study of environment, as separated from the genetic equipment, derive from the fact that a certain environment may be a factor of selection attracting those with a certain kind of genes; or those with a certain genetic equipment may favor, and therefore seek, a special environment. Thus, a mutual interaction between the two factors does occur. This may be true in the case of the bus drivers and bus conductors in the London transport system, in whom Morris[2] found different mortality rates from coronary heart disease in the two groups. His guess was that the difference was due to the environmental conditions of their occupation, but he later found out, by studying the waist size at the time of their application for jobs, that the two groups originally were of different body-build, each of which may deliberately have chosen his type of job and, thereby, his environment. This may illustrate the even greater difficulties in interpreting statistical differences between samples of populations in various countries.

Various Aspects of Physical Environment

Leaving aside the genetic aspect for the moment, I shall turn instead to the role that *physical* environment plays in the development, and subsequent course, of some cardiovascular disorders. Some influences may be of short duration but great intensity, others may be of lower intensity but exert their effects over long periods of time. Let me mention a few examples.

Intrauterine environment. Before we breathe any air at all, we are enclosed in a low-pressure, or rather low-oxygen, chamber, namely the uterus, which is our first environment. This is a place where the oxygen tension in our blood may correspond to that at 30,000 feet altitude, where an adult is said to lose consciousness after 30 seconds and to die within a few minutes. In this frustrating environment, the human fetus not only lives but also puts on weight and passes through its whole amazing development, ending in a brief parachute trip down to atmospheric pressure at birth.

It is no wonder that the fetus is vulnerable to *additional* environmental strain. Certain virus infections, rubella in particular, that affect the mother in her early pregnancy, may give rise to congenital malformations of the heart in the child. Congenital heart disease, however, apparently derives also from genetic factors; siblings affected with such malformations are by no means uncommon. During the Australian epidemics of rubella in 1950, patent ductus arteriosus was eight times as frequent among the congenital heart lesions as in material from non-rubella periods[3].

Among other things to consider about the fetal heart, is the effect of x-ray irradiation. This, of course, may affect both the genetic material of the mother and the developing organism of the child. Recent studies[4,5] throw some doubt upon the prevailing concept of the lack of insensitivity of the adult heart to irradiation and, thus, give increased strength to the present tendency to a more careful use of x-rays in general. With regard to cardiology, I think angiocardiography should, if possible, be avoided in pregnant women.

Climate. Climate sets the rules for vegetation, animals and microorganisms. In so doing, it may expose the unprotected man to some plagues that may result in cardiovascular disease, such as streptococcal throat infections in some areas or cardiac trypanosomiasis (Chagas'

disease) in South America. Generally, though, we have found means to deal with many climate factors, both by "acclimatization" and by the use of various protective devices.

It is difficult to evaluate the often presented evidence on the alleged influence of rapid barometric or temperature shifts on blood pressure, on attack-rate of angina pectoris and of myocardial infarction. More convincing are recent experimental studies by Burch in New Orleans[6] on the effect of heat on the heart, in normal people and in those with congestive heart failure. His data show that the heart performs relatively more work when the subject is in a hot and humid environment. The heart in congestive failure is not able to increase its work output as efficiently as the normal heart. In a hot and humid climate, mere bed rest may be associated with considerably increased cardiac work. The importance of protection against heat and humidity in the case of cardiacs, both at work and at rest, is obvious.

Cold seems to be less of a problem than heat: protection is accomplished with greater efficiency. Nevertheless, our earlier concept of temperature ranges compatible with life in recent years has been modified not least by studies related to the use of hypothermia in surgery. In 1950, it was shown by Luyet[7] that chick embryos, or even excised embryonic hearts, could be immersed in liquid nitrogen at -195° C. for hours, and despite this treatment resume cardiac activity after rapid rewarming. Later on, workers like Andjus and Smith[8, 9] succeeded in cooling rats close to zero and hamsters to temperatures even below zero with successful reviving, for a period of time after rewarming. In preparation for cardiac surgery, dogs have been successfully cooled to temperatures of about 16°- 18° C. with survival. There is, however, some evidence that such low temperatures inflict damage upon the myocardial cells. Temperatures around 25°C., or even less, have been used in human cardiac surgery. The occurrence of ventricular fibrillation seems to increase as the temperature goes down, and surgeons now prefer to operate in hypothermia at temperatures at just below 30° C. The tendency to ventricular fibrillation may be counteracted by drugs, such as procaine amide, but it has recently been shown, that a couple of weeks in a sub-zero arctic climate may result in an acclimatization process in dogs, which, likewise, affords strong protection against ventricular fibrillation during subsequent exposure to hypothermia[10]. It is believed that this acclimatization is not intrinsic to the heart itself,

but due to other mechanisms. This would, then, be in accord with what is known about hibernators, in whom the fundamental condition for tolerating body temperatures of only a few degrees centigrade for almost any length of time, seems to rest in the ability of their nervous system to lose its specific sensitivity to external cold. From comparisons that we have made between the electrocardiograms of hibernators and of patients operated upon in hypothermia, an impression is gained that the myocardium of hibernators has a special capacity to endure retardation of electrical processes without developing such irritable "trigger" areas as appear in dog and man[11].

Atmospheric pollution. One sometimes feels that Western civilization is too severely blamed for the harmful environmental effects that it creates. As regards pollution of the air, however, I think the accusation is valid. Certainly the air was much cleaner in the days before industrialization and the development of big cities. The immediate effect on mortality of heavy "smog" periods seems to be established beyond doubt, and many of those who succumb probably suffer from cardio-respiratory insufficiency.

The step from "smog" to "smoking" perhaps seems to be a giant one. But smoking, particularly the deep inhalation of cigarette smokers, seems to be related to an increased incidence, not only of cancer of the lung, but also of coronary heart disease. In the acute experiment, some people give evidence of rather strong reactions in the cardiovascular system to nicotine inhalation, and it seems reasonable to suppose, that repeated and prolonged exposure to such influences will eventually result in organic damage. However, the link between smoking and heart disease need not necessarily be a direct, pharmacological one. There may be a common denominator for both coronary heart disease and the habit of smoking.

It is also well known, that the spread of streptococcal infection or tuberculosis, both of which may give rise to heart disease, is facilitated by crowded living conditions. This is a field where preventive medicine has been particularly successful.

Nutrition and exercise. These are both related to physical environment. In underdeveloped countries, where choice is limited, nutritional deficiencies may give rise to heart disease such as beri-beri heart, and, perhaps, endomyocardial fibrosis. In our civilization, much more is left to the choice of the individual with regard to nutrition and exercise and

they are less intimately connected with the physical environment. I will return to these problems later on.

Psychologic Environment

Mechanisms. To many of us the concept of environment is rapidly losing its physical meaning and is translated into more or less psychological terms. Even such e' .nents as are obviously physical, as inhalation of nicotine and carbon monoxide, may be considered by some chiefly as expressions of a deeper mechanism. With the prevailing interest in psychology we are sometimes apt to forget that psychological influences are created and conveyed to us entirely by physical means; the reception of energy by our sense organs and the elaboration of the corresponding signals by the brain. In this sense, mental stress from environmental influences depends on the intensity of the stimulation received, and of its significance to the cerebral computer. Thus, impulses from the environment converge to the brain and enter into an immediate relationship with the continuous flow of information that the brain receives from the body itself. At these cross-roads, the impact of the environment upon the heart and circulation becomes imminent, for every change on the afferent side affects the efferent flow in the autonomic nervous system. An important center of coordination has been found in the reticular formation of the brain stem, which does not only permit simple types of reflexes but is concerned with selection, suppression and integration of afferent impulses. This area is concerned with the regulation of wakefulness and sleep and the adjustment of autonomic functions to changes in environmental demands. Very interesting studies are now performed in a joint approach of neurophysiology and animal psychology, to demonstrate the ways in which situations of "responsibility", "frustration" and so on give rise to "psychosomatic" responses. But also stimuli of a more simple type, like ringing of bells at short intervals, for considerable periods of time, may give rise to reversible hypertensive heart disease in rats.

Culture. When psychologic environment is discussed in relation to disease, one will often think of conflicts in the mind, extended tension, or a general inadequacy in dealing with a situation. But the social and cultural frame-work of our daily living—the things we sacrifice in order to achieve others—is of equal importance. In times and places where a full day's manual labor was barely enough to provide food

and shelter for the family, there was not much choice in patterns of behavior. To-day, the economic situation of a great many citizens in Western societies gives them considerable opportunities to choose their mode of life. Nevertheless, few of us step outside of the circle drawn by our culture. Accepted standards of behavior determine to a great extent the amount of physical exercise that we perform, the rest we allow ourselves, and our habits of food intake, drinking, smoking and overt emotional outlets.

The individual patient may be advised to take more exercise, or eat less fat or quit smoking as a *therapeutic* measure. For persons *at risk* such recommendations may be given prophylactically. A very important task will be to determine *who are at risk* and how large this group should be made. To change the cultural setting for *people in general* on behalf of preventive medicine is probably beyond anybody's capacity.

Conflict and mental stress. Few people escape becoming involved in situations of acute conflict or chronic stress. No one has more ably analyzed the effects of the psychologic environment in the genesis of "psychosomatic" diseases than Harold G. Wolff[12]. I believe that many of the manifestations of cardiac neurosis (anxiety neurosis with circulatory disturbances or neurocirculatory asthenia), do belong here, and similar mechanisms are probably responsible in a great many patients with arterial hypertension. For the precipitation of anginal pain in many people—even cardiac infarction in some—and of various kinds of cardiac arrhythmias, psychogenic factors, of threatening significance to the individual, also appear to act as triggers.

Both individual case records, and data collected from physiological experiments on humans have demonstrated the possibilities and partly also the pathways over which the psychologic environment affects the circulation in acute situations. As far as chronic diseases go, there is always some difficulty in proving that the accumulation of acute effects actually results in permanent damage.

One way of investigating this problem would be such longitudinal studies of individuals as have been performed by Hinkle and Wolff[13], and others. In their studies, the incidence of manifest disease, or even latent disorders, has been related to personal events during the period of observation. Hinkle and Wolff have stated that "difference in exposure to physical hardships, dietary differences, differences in exposure

to infections or toxic agents, and differences in general physical life experiences did not appear to account for the differences in susceptibility to illness". They found that "adverse childhood experiences, difficulties in adult interpersonal relations, and heavy demands from the social environment are positively associated with an increased susceptibility to illness *of all forms*." This includes both "organic" and "functional" diseases; there is no sharp dividing line between the two because "functional" disorders of course are "organic" in a molecular sense, and even what appears to be "organic" is subject to the dynamic change of body constituents. All goal-directed reactions of the brain—conscious or subconscious as they may be—to adapt the bodily functions to the environment, including the stressful environment of a frustrating situation, will affect both the somatic nervous system, the autonomic nervous system and endocrine functions. Hinkle and Wolff, furthermore, believe that "man's relation to his social environment has relatively small influence upon the *form* which illnesses will take but has a major influence upon the time and the situation in which illnesses will occur and the course which they will pursue."

From the practical standpoint of the physician, it is important to know whether "adverse childhood experiences" or "difficulties in adult interpersonal relations" or "heavy demands from the social environment" are operating in the individual patient. Many internists and cardiologists, dealing with heart patients, probably focus their interest upon the *present* situation and try to identify and eliminate the acute stress-provoking agent. This is probably just about what the ordinary practitioner *can* do for his patient.

However, much of what we learn from the present history of some patients with "functional" troubles may be a repetition of cardiovascular—and perhaps also biochemical—responses to earlier experiences (maybe those in childhood), which have been in a way conditioned to overt or symbolic dangers of a certain type. Such conditioned responses are: rapid and shallow breathing with a flattened and immobile diaphragm, frequently encountered in neurocirculatory asthenia; changes in cardiac rate and rhythm, and of stroke volume and exercise tolerance as well; changes in peripheral vascular tone with concomitant variations of blood pressure; and possibly also changes in water metabolism, viscosity of the blood, and of some coagulation factors. It is tempting to interpret bodily symptoms in terms of expression of psychological attitudes, and

this is frequently done. It is, however, a field where I think one should be very cautious in separating theory from fact. Inasmuch as environment here plays a role, part of that role belongs to the past, and it may require great efforts to replace old patterns with new and better ones. Nevertheless, practical attempts to treat patients of this kind by drastic "rehabilitation" of their whole environmental situation sometimes offer a better chance than trials with drugs or even verbal psychotherapy alone.

Environment in Relation to Certain Chronic Diseases of the Circulation

I have thus far tried to discuss some of the environmental influences that may affect the cardiovascular system. When we consider the different heart diseases, these can be divided into two categories. One is the group in which specific infections, such as rubella in the mother, hemolytic streptococcal throat infections, tuberculosis and syphilis, all represent clear-cut etiological factors derived from the patient's environment. Such factors can be dealt with by means of classical preventive methods, directed against the spread of the agent, or prophylactic treatment at an early stage of the disease.

In other disorders of the circulation, however, such as essential hypertension and coronary heart disease, it is much more difficult to differentiate between genetic factors and environmental ones. This is so because the dividing line between normal and abnormal is, to a certain extent, arbitrary, "subclinical" manifestations may escape notice, and "sick" versus "healthy" becomes a matter of quantity rather than of quality. In such a situation, "environment" may be responsible for the *degree* to which the genetic potential may unfold itself. Apart from intrinsic dynamics, the course and prognosis of such diseases depend upon the extent to which the individual is subjected to undue demands from the environment, or can rely on its support.

Heredity and environment. With regard to heredity versus environment, Pickering[14] deserves the credit for having attacked this crucial problem in essential hypertension by asking: What is the *size* of the genetic factor? Only when we have determined the role of the inevitable genes will it be possible to assess the environmental factors, and perhaps, modify them. Even when treated by this eminent scientist, the present knowledge remains rather meager, as shown by the following

quotations: "So far as children are concerned, the point would therefore seem to emerge that, as compared with environment, heredity is of considerably less importance in determining arterial pressure than in determining height and weight. Unfortunately, there are no comparable figures for adults, but . . . [certain data quoted by Pickering] suggest that the relative contribution of inheritance decreases, while that of environment increases with age." Pickering also attempts to weigh the factors behind casual blood pressure levels and says: "It would be no more than a guess to say that the genetic factor and diurnal variation together account for half of the deviations of arterial pressure from the norm encountered in the population at large. *This would leave half to be accounted for by environmental factors.* I venture to make these guesses, not because I have any grounds for believing they are, even approximately, correct, but to draw attention to the necessity for obtaining data from which more accurate estimates can be made."

There are, of course, certain inborn errors of metabolism, such as familial hypercholesterolemia, in which the genes account overwhelmingly for the fate of the patient. Nevertheless, environment may become as important,—with progress in biochemistry one will find that faulty pathways of metabolism can be by-passed one way or another. In biochemically well-defined diseases, familial occurrence means that the cause is genetic. But with other conditions, obesity for example, familial occurrence may have quite another significance and the common denominator may be of cultural, or otherwise environmental, origin. In such families, the cook-book rather than the genes may be inherited. I do not say this to minimize the influence of the family-factor in some cardiovascular diseases, for there is ample evidence in life insurance statistics of its prognostic importance, but I think it is pertinent to challenge the idea, that familial occurrence of disease necessarily is genetically determined and as such is inaccessible to any therapy. Part of the familial occurrence may be environmentally determined, and the genetically determined part may, eventually, become accessible to biochemical treatment.

Geographical incidence of heart disease. During the preparation of this lecture, I have been looking into quite a number of papers on the geographical distribution of heart disease. One cannot help being disappointed with the information available

from this source. It may be based on data from official vital statistics, or from life insurance studies or from hospital surveys or, perhaps, from autopsy material. It does tell something, of course, about that sample of observations, but it cannot be used without qualifications to explain the effect of environmental differences on the type and incidence of heart disease.

One of the best records of the geographical pathology of heart disease that I know of, is Snapper's well-known book[15] on *Chinese Lessons to Western Medicine*. While reading it again this year, I could not but admire the wisdom in his interpretation of his clinical observations, especially in the light of recent scientific knowledge. In Snapper's experience with heart disease in North China, rheumatic heart disease was frequent in clinical and autopsy material, despite the infrequency of the classical type of rheumatic fever as it was known then in the Western world. Furthermore, he found arteriosclerosis to be infrequent, while hypertensive heart disease, even with relatively low blood pressure levels, was common. As to rheumatic heart disease, similar views have been expressed by Padmavati[16], of India; while the marked frequency of hypertension and cerebral hemorrhage in Japan (as reported by Kimura[17]), seems to go well together with Snapper's Chinese data. It is interesting to note, that Snapper in 1941, as a possible explanation of the infrequency of atherosclerosis in the Chinese, mentioned that the "Chinese diet contains only small amounts of cholesterol but considerable quantities of unsaturated [fatty] acids, especially of linoleic and linolenic acid." This brings us straight into the center of the present discussion of unsaturated fatty acids.

The statements of experienced clinicians such as Snapper's from China, and Paul D. White's from Italy and other countries, are, in my opinion, of great value although they may be subject to statistical criticism. The World Health Organization publishes annually a large volume full of data from all parts of the world on causes of death. It is quite obvious that death certification in cardiovascular diseases is at times very uncertain, and depends on the professional standards and diagnostic nomenclature of those who issue the certificates. For example the introduction by James Herrick, in 1912, of the clinical picture of coronary occlusion, has had tremendous repercussions on our concept of disease and of cause of death, but the penetration and acceptance of this concept within the medical profession of the world has neither

been a rapid, nor a uniform process. Thus, in comparing mortality data of various countries, of various social groups and various periods of time, the performance of the doctor who issues the death certificates enters as a deciding "environmental" factor.

Obviously, there are a number of assumptions to be controlled before any definite conclusions can be drawn from such international comparisons. It is difficult, probably impossible, to check them all at one time. This is the reason why some of these studies are being extended to so many parts of the world. What such epidemiological studies can contribute is to check the validity and comparability of international data on mortality, morbidity and biological characteristics in populations and this is a fundamental task for the study of environmental medicine. It may also provide some hypothetical clues to further research. But even when available data indicate a fair correlation between the incidence of disease and some apparently independent variable, there always remains the question, whether this correlation is more than coincidental; whether both variables have a common denominator, or whether A is the *cause* of B. The proof of the causal relationship will, I believe, have to be experimental and will consist in demonstrating the mechanisms by means of which A results in B. No one can deny that the diet-hypothesis of coronary heart disease has put many research teams on the track in this direction.

What Do We Know About the Increase in Coronary Heart Disease?

There is apparently a common belief, that coronary heart disease and myocardial infarction are rapidly increasing in the population, not least among relatively young people and that is why one has started to look for environmental factors in this disease. Mann[18] has recently remarked that removal of other causes of death would be expected to account for a part of the apparent increase in coronary heart disease. Lew[19], in a critical analysis of vital and life insurance statistics, felt that in the United States there has been "a moderate increase in the mortality (from arteriosclerotic heart disease), of white males over the last 15 years (probably less than a fifth of the recorded increase being real), and little, if any, increase in the mortality among white females." According to this view—which may be somewhat exaggerated—most of the increase observed is due to changes in reporting and classifica-

tion of deaths by cause.

There are, however, other aspects than mortality figures. Morbidity data also may be of interest. During the last few years we have been studying what is happening with regard to myocardial infarction in my home town of Malmö, the third largest city in Sweden[20]. There, we have a rather favorable situation for such studies. There is only one large general hospital for all of the 200,000 inhabitants of this city. The percentage of autopsies performed on patients who die in the department of medicine has, for more than 20 years, varied between 90 and 95 per cent. We could hardly have had a better set-up in which to work. Nevertheless, interpretation of our data must be done with great caution.

It is quite clear, that there has been a three-fold rise of myocardial infarcts in the *hospital* material from the middle of 1930 to the end of 1940. After that time, there seems to be no further rise in incidence. Here, we have a homogenous population and there is no change in the genetic factors. The changes must have another cause. We have been investigating the composition of the material of hospital infarcts in order to find out if we could trace any well-defined influences that might have been operating. As far as we can see, neither the availability of hospital beds, nor the composition of the infarct group with regard to sex ratio (3:2) or infarct severity has changed in any marked way. However, the mean age at first infarction has shown a constant upward tendency. In the majority, myocardial infarction appears to be a disease just before or around pension age—67 years. This increase is not sufficiently explained by a greater number of old people in the population. If it is a true increase, it seems to have affected the total population. If it is a spurious rise, there must have been an increased awareness among the practicing physicians of the diagnosis of myocardial infarction, and a greater willingness or perhaps, nowadays, eagerness of the patients to go to the hospital. From a simultaneous check of the death certificate material, it is clear that such factors may influence the composition and magnitude of the hospital material considerably. The myocardial infarct in a sense represents that part of the iceberg that we can observe. Coronary heart disease without manifest infarcts, that is below the surface, is hard to estimate. A change in specific gravity may affect both the parts of the iceberg above and below the surface.

Occupation and coronary heart disease. In our material, there was in the later years a tendency to higher infarct morbidity in the employer group than in clerks and workers. In British studies (particularly by Ryle and Russel[21]), the observation was made that mortality from "angina pectoris" was highest in the upper social class. Several American investigators, however, have been unable to confirm these observations in their studies. Both Stamler[22], in Chicago, and Lilienfeld[23a], in Baltimore, have reported opposite findings. Thus, in Baltimore the lowest death rate from arteriosclerotic heart disease at ages below 65* was found in the highest socio-economic groups, and the same was true for Chicago.

From life insurance studies, Lew has identified a variety of occupations with a significantly higher mortality from heart disease. These include stationary firemen, janitors and porters, policemen, barbers and physicians. It is hard to find a *single* common factor in these occupational groups. To a certain extent, excess mortality of sedentary groups, like that of urban inhabitants over rural people, may represent a selection factor. In other words: does urban life, or certain occupations, *attract* people with heart disease or "predisposed" to heart disease—or does it "*cause*" heart disease?

This also brings up the question: What is the real significance of occupation or social class with regard to environmental influences in our present civilization? Many factors may be enumerated in this connection: 1) genetic selection; 2) mental stress; 3) various degrees of physical work load; 4) quantity and composition of food; 5) habits of smoking and, perhaps, of alcohol consumption, etc. Morris has recently remarked, that "the ready-made social groups which have been such a useful way-in for epidemiological research in the past

* Hardin Jones[23b] has proposed some very interesting theories on the relationship of aging to life expectancy in various countries and at different times. He believes that the process of aging is hastened by any previous disease experience. Such "increments of physiologic deterioration" seem to have more serious consequences for longevity, the earlier they occur in life. Thus, childhood experiences are the most important factors determining longevity. This brings the factor of *environment* much further back than we are used to think of in somatic diseases, at least. This concept, as applied to various populations, leads to a mathematical discrimination of chronological age and physiologic age, and in Jones' opinion, the peoples in Scandinavia and the Netherlands are at least six years younger physiologically than those of the United States and Finland. Corresponding differences may exist between socio-economic groups and may affect comparisons in which chronologic age is used in defining the groups. I am not in the position to judge the validity of Dr. Jones' theory, but the more thought I have given to it, the more I think it has got something. The increase in mean age of our infarct patients may be an expression of a more favorable physiologic age for the later groups as compared to the earlier ones. We have seen something of the same kind in our patients with rheumatic heart disease, in whom life expectancy in the conservatively treated group improves alongside with the prolongation of the life expectancy in general. But, I admit, it is hard to say in what period of life environmental influences are most important because Scandinavian wartime experiences indicate that mortality rates for atherosclerotic heart disease may change rather abruptly.

may be less helpful in the future, as concerns shifts to such matters as smoking, exercise and diet—say its fat content in a country with full employment and high earnings." This is definitely so. A survey of food habits in south Sweden[24] recently disclosed that the upper social class ate more margarine and less butter than did the laborers. I think it is fair to state, that the relation of occupation to mode of living is a very complex problem indeed, and one has to be very careful in drawing inferences.

Some Special Environmental Factors

Mental stress. The concept that mental stress is of particular importance for the development of coronary heart disease derives from several sources. One is probably the feeling that such stress is more frequent nowadays than heretofore, and consequently, is a feature of the particular "environment" in which hardening of the arteries occurs. Another source was the earlier observation of an especially high incidence of such disease in upper social groups. It is relatively easy to see the physiological connection between mental stress and the discharge of epinephrine and norepinephrine, with concomitant loads on the circulation and sometimes extreme physiological variations in circulatory response, as in neurocirculatory asthenia. Furthermore, a previously damaged heart may suffer, and many instances of sudden death have been attributed to such a mechanism. A recent paper has even drawn attention to the potentially lethal effect of ward rounds on patients hospitalized for myocardial infarcts!

However, I think Howard Sprague[25] is right when he maintains, in an editorial in *Circulation*, that convincing proof is still lacking that mental stress is instrumental in the genesis of atherosclerosis. This is also the preliminary conclusion of the epidemiologists. In the Albany study[26] neither job classification, nor self-assessed ratings of "tension" could be related to the age-adjusted incidence of ischemic heart disease.

There is, however, one occupational group under mental stress where an excess mortality from coronary heart disease seems to be established in several countries, namely in physicians. Furthermore, Morris has found that the British general practitioner is worse off than the specialists[27]. It is hard to know yet, what factors are operating here, but lack of adequate rest may be one of them and I believe that this aspect

ought to be studied more carefully.

Exercise. There has been considerable argument concerning the unfortunate effects on the coronaries of too little physical exercise. Morris presented an argument in favor of this idea with his studies of the bus drivers, who had twice the infarct rate of the bus conductors, but Keys and his group[28] were never convinced, and when Morris began to measure the size of their uniforms, he became less sure of himself. The Los Angeles group under Chapman[29] did not feel convinced that the physical demands of jobs had any significant influence on the occurrence of coronary disease in the large group of civil service employees whom they were investigating.

Food. So much has been said about food habits and atherosclerosis, particularly from geographical comparisons by Keys and his group, and so much is still to be revealed about the *true* mechanisms of fats, proteins and calories in relation to serum lipids, and serum lipids to cholesterol deposits in arterial walls and to the process of thrombosis. Animal physiologists have found that rats that are kept on a meager diet when young, live longer than those which have had plenty to eat throughout their lives. Observations on serum cholesterol and incidence of atherosclerosis in two groups of Yemenite immigrants to Israel[30], one arriving over 20 years ago and the other group in recent years, indicate that cholesterol values and atherosclerosis mortality were significantly higher in the former group than in the latter. This was related to their income level. In those parts of the world, where fat intake was reduced during World War II, there was an immediate decline in over-all mortality, and not least in cardiovascular diseases and diabetes[31]. Some workers maintain that this was an effect of a reduction in the degree of atherosclerosis, insofar as this is a dynamic process, capable of going both ways. Others, as Strøm and Jensen in Norway[32], seem to believe that the factors operating may have something to do with a reduced tendency to blood clotting and thrombosis in general.

Personally, I feel that the linking together of coronary heart disease and the diet is the most convincing of the environmental "clues" so far obtained. One shall, however, have to look at it both with regard to atherosclerosis and to thrombosis. I would hazard a guess that the thrombosis-part of the problem may be easier to separate experimentally in man than the atherosclerosis-part.

International Studies

I have been taking your time in analyzing some of the inherent difficulties and controversial findings with regard to environmental factors in the etiology of coronary heart disease. In the end, one may find it difficult to express *any* opinion on the subject. It is not that we are without "clues", or even "proofs". Rather, we have too many of them and we try to explain the situation in terms of "multiple causation", for whatever such a phrase is worth.

A study group on atherosclerosis and ischemic heart disease, which worked in Geneva during 1955 and which has recently published its report (in the World Health Organization Technical report series[33]), has put forward some basic recommendations for future research. That more facts are needed is easy to understand, when one considers that there are perhaps ten times as many articles on the subject of the artificial heart in the current literature as there are on environmental factors in heart disease (other than fat). In a fortnight, another such WHO group will meet in Copenhagen to map out practical measures in this direction. Prospective studies like those at Framingham and Albany, to my mind, represent the most hopeful approach so far, besides the experimental shortcuts made by lipid chemists. I have no doubt, that five years from now we shall have much better evidence available to back up practical recommendations than we have as yet.

Summary

When I set out to prepare this paper, I asked myself the question: What can we accept as reasonable evidence of environmental factors in the development of cardiovascular disorders? What I had hoped for, was to arrive at some recommendations about what we as physicians can do about environment, in preventive or therapeutic terms. However, in the analysis that followed, I soon found that one cannot take apart the man and his environment and treat them separately. Man carries an image and a part of his environment within himself, and he cannot live without it. If that environment incorporated within him gives him trouble, one can ask: Is there any hope of remodelling environment, or can anything be done to make the human organism more fit to tolerate, handle and dispose of noxious elements of environment?

Some environmental influences are so well-known and so obvious that I have dealt only briefly with them. In this category I place the various kinds of infections that give rise to inflammatory processes within the heart and the vessels, such as rheumatic and syphilitic heart lesions. In other instances, pulmonary heart disease may become a late result of chronic affections of the lungs, such as pneumoconiosis, and perhaps even chronic bronchitis, a disease that seems to be a particularly frequent cause of invalidism in Great Britain. In both these types of heart disease, there are classical methods of prevention. Spread of infection can be curtailed, and early infection vigorously treated so that the battle between agent and host does not result in chronic disease.

In the other types of cardiovascular disease, as represented by hypertensive heart disease or coronary heart disease and perhaps some conditions attributed to "neurocirculatory asthenia", the direct relationship between environmental factors and the resulting disorder cannot be traced so easily. There has been a popular belief, inspired by ourselves, that coronary heart disease is a particular feature of our present Western civilization; that it is markedly on the increase, that those who have heavy responsibilities and a mentally stressful life are especially at risk and that those who eat much, especially much animal fat, expose themselves to great dangers.

I have tried to examine some of the information brought forward to prove the case. It is clear that these diseases *are* a great problem of public health today, but I think we must be very cautious in assessing how big the real increase in morbidity is as compared to earlier years. Data from the past are always difficult to interpret, but if we can grasp the present trend by *prospective* studies we may be able to extrapolate backwards and arrive at a better guess than is possible at present. It is clear that sex and age, possibly also race and perhaps the somatotype are of importance for the development of the disease. We cannot do much about these things. With regard to the type of occupation or the way of living—which may be subject to change—evidence is conflicting, and because so many environmental factors are culturally inter-related, it is as yet difficult to tell them apart. Nevertheless, whether one believes that environmental factors are important or not, it would seem as if periods of rest for the mind, a fair amount of physical exercise, and less smoking would be safe recommendations. In the "fat and calories" discussion we are left with two proposals: one,

to reduce our diet, the other, to add even more fat, in the form of essential fatty acids[34].

One can then ask: Can we arrange life in our society so as to allow more peace for the mind, more physical exercise, less smoking and, perhaps, less food or less food of the kind we are used to now? Or, shall we accept environment as it is and instead offer more tranquilizers and preventive psychiatry (whatever that is) and some enteric-coated enzymes to detoxify the food we like to eat? *If* we believe that certain environmental factors in hypertensive and coronary heart disease are the deciding ones, then these are the crossroads at which the medical scientists and the medical profession stand today. Here, the classical preventive medicine can offer only little help. Even if it enlists the so-called Health Education movement, the task of re-educating a civilization on behalf of its future health seems overwhelming. If such a re-education will be possible at all, it must occur together with a reorientation of technology, economy and cultural values in general, and can certainly not succeed against the basic trends in the technical evolution. Before any changes are advocated, one must have very definite proofs of the *causal* relationship between the environmental factors and heart disease, and of the usefulness of proposed changes. As yet, we are hardly in that position, and we still have to await the results from investigations designed to test the various hypotheses that have derived from the present phase of the growing interest in environmental medicine.

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OBSERVATIONS ON THE NATURE OF STAPHYLOCOCCAL INFECTIONS*

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DURING the past decade, staphylococcal infections in hospitalized patients have gradually emerged as one of our more distressing therapeutic problems. Recurrent outbreaks of staphylococcal disease are now common in nursery units where newborn infants are congregated. Important surgical procedures are occasionally compromised by serious staphylococcal sepsis. Staphylococcal infections are a common terminal event in the course of debilitating medical illnesses which require hospital care[1].

It appears progressively clear that our present problems with this microbe reflect a shift in the nature of infections which can produce life-threatening disease in medically sophisticated communities today. Before the development of antimicrobial drugs, acute bacterial infections were a major cause of human illness and death. Here the contest was explosive and the outcome was determined in a relatively short span of time—either the patient died or the infection was controlled and eradicated by the host.

As these infections have come under the control of antimicrobial agents, certain infections caused by microorganisms commonly residing within most normal human beings have emerged to prominence and may be increasing in incidence. Infections caused by staphylococci, certain enteric bacilli, and fungi—all normal inhabitants of normal humans—are now common in our hospitalized patients.

These microbes and the infections they produce differ in certain important characteristics from the acute bacterial problems of yesterday. All commonly produce less decisive, focalizing disease in which

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both the human host and the bacterial parasite can survive together for long periods of time. Classic humoral immunity does not arise, or if present, does not appear to contribute materially to recovery. Perhaps of most significance, these microorganisms *appear to require local or systemic alterations in host resistance before progressive infection ensues.* Indeed, these microbes might be considered the jackals of the microbial parasite world: unable to mount an attack against normal humans, they commonly invade in other disease states where host resistance appears crippled or compromised.

There is much evidence to suggest that host factors play a more important and more complex role in such endogenous infections than in acute bacterial disease. In the case of staphylococcal infections, the status of the skin, the reticuloendothelial system, the bone marrow, or other host tissues has seemed of more importance than the *virulence* of staphylococcal strains in determining whether serious clinical infection will occur. Our increasing intrahospital difficulties with staphylococci appear, in certain measure, to be caused by the disease states which require hospital care, or by the things which we do to patients in modern hospitals which alter human resistance.

Operating on this thesis, we have focused our experimental attention on the ways in which host tissues deal with experimentally induced staphylococcal infections. This evening I should like to present certain observations derived from these studies which tend to set the staphylococcus apart from other gram-positive cocci with which it is frequently grouped. Recognizing the pitfalls of extrapolating from animal experiment to human disease, these experimental facts allow some tentative guesses about certain peculiar features of staphylococcal disease in man, and can perhaps form a background for the problems surrounding treatment to be presented by Dr. Walter*.

The behavior of staphylococci within phagocytic cells. Some years ago we noted that pathogenic staphylococci were steadily ingested by human leukocytes in systems in which other virulent microbes failed to be phagocytosed. Despite the fact that virtually all staphylococci were ingested by leukocytes, little or no killing of staphylococci could be detected. Subsequent studies indicated that certain staphylococci could survive for long periods within both human and rabbit polymorpho-

* Paper on *The Control of Staphylococcal Sepsis,* presented later at this same session by Carl W. Walter, Associate Clinical Professor of Surgery, Harvard Medical School.

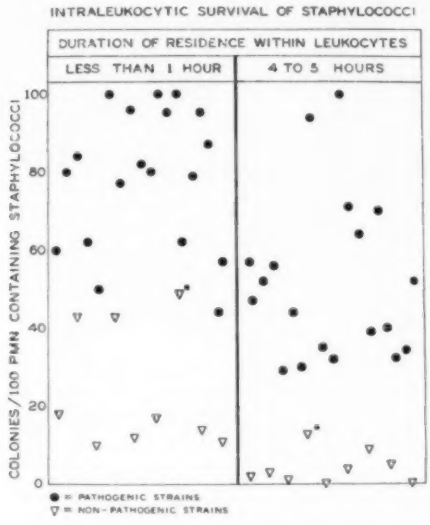

Fig. 1—Strains of coagulase-positive staphylococci isolated from infection survive in leukocytes for long periods. Strains of coagulase-negative staphylococci obtained from the body surfaces do not.

nuclear leukocytes and that this ability correlated well with human or animal pathogenicity[2].

As shown in Figure 1, strains of coagulase-positive staphylococci isolated from human infection invariably survived for four to five hours within the interior of polymorphonuclear leukocytes. In contrast, coagulase negative strains isolated from the skin, air, or anterior nares were almost uniformly killed within leukocytes during this time period. A sharp differentiation could thus be made between these two broad species of staphylococci.

Subsequent studies by Tompsett[3] showed that such pathogenic staphylococci could also survive within mononuclear phagocytes obtained from rabbits while non-pathogenic strains did not. These studies have since been confirmed by others[4].

Recent studies have attempted to determine whether such intracellular survival modifies the nature or course of experimental staphylococcal infection. We now believe that it can and does.

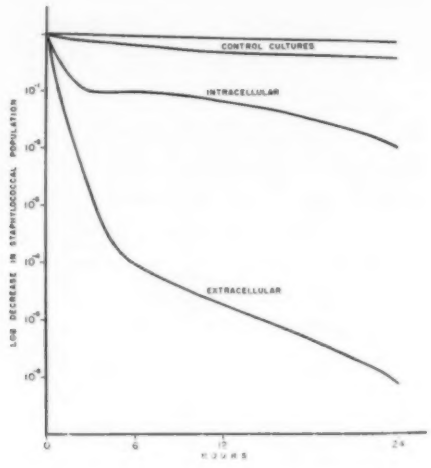

Fig. 2—The protection of intracellular staphylococci from penicillin (redrawn from Tompsett, reference 5). Intra- and extracellular staphylococci survive in *in vitro* systems. Extracellular staphylococci are 50 to 200 times more susceptible to destruction by penicillin.

First, intracellular residence may protect staphylococci from substances in extracellular fluids which ordinarily destroy them. The studies of Tompsett indicate that intracellular staphylococci are protected from antimicrobial drugs present in the surrounding milieu. From 50 to 200 times the amounts of penicillin or streptomycin necessary to kill extracellular staphylococci are required to appreciably affect intracellular staphylococci[5].

Such an experiment is shown in Figure 2. Control cultures of staphylococci residing extracellulary or intracellulary survive for long-time periods. However, when antimicrobial, in this case penicillin, is added in amounts which produce rapid and significant killing of extracellular staphylococci, little change is noted in staphylococcal populations residing within human leukocytes. Thus, as is the case with Brucella[6] or tubercle bacilli[7], incorporation within cells appears to offer the staphylococcus a protective cocoon in which it can survive in unfavorable climates.

It appears clear that this protection cannot be explained by simple

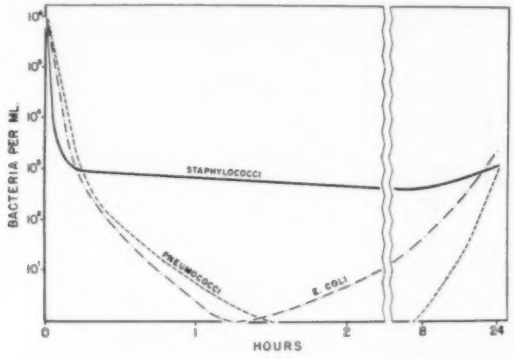

Fig. 3—The blood stream clearance of three different bacteria. Initial disappearance rates are similar. Only staphylococci persist in the circulation for long periods (see text). Figures 3-6, inclusive, from Rogers, D. E., Cellular Management of Bacterial Parasites, Pasteur Fermentation Centennial 1857-1957; a Scientific Symposium, pp. 61-73, 1958, reprinted by permission of Chas. Pfizer & Co., Inc.

failure of antimicrobials to reach the interior of leukocytes. What seems more probable is that staphylococci are metabolically less active within cells and that this sluggishness conditions their susceptibility to antimicrobial drugs. We will have more to say about this later.

Intracellular survival may also modify the way staphylococci behave within the vascular system of experimental animals[8]. When large numbers of staphylococci are injected into the rabbit blood stream, the majority of microorganisms are removed during the first 10 to 15 minutes by the liver and spleen. Then something happens abruptly. The splanchnic tissues no longer trap staphylococci, and a prolonged bacteremia lasting for hours, indeed for many days, results. As shown in Figure 3, this is not the case with bacteria commonly destroyed within phagocytes. Pneumococci or *E. coli* initially disappear from the circulation even in fatal infections in which bacteremia subsequently returns.

It is our current belief that staphylococci surviving within polymorphonuclear leukocytes may play a role in this surprising persistence of microorganisms within the blood stream.

During the initial period of rapid blood stream clearance of staphylococci or pneumococci, polymorphonuclear leukocytes almost disappear from the circulation[9]. As shown in Figure 4, leukocytes then re-

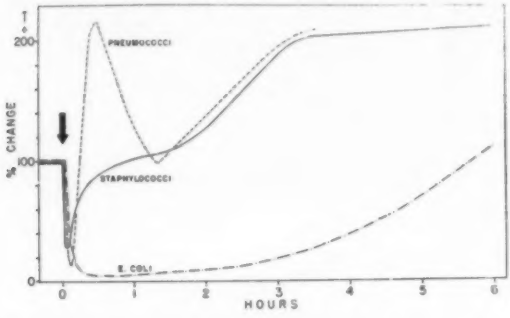

Fig. 4—Changes in the number of circulating granulocytes following intravenous injection of three different bacteria. Injection of staphylococci or pneumococci results in rapid disappearance and reappearance of circulating leukocytes. Injection of *E. coli* produces a prolonged granulocytopenia. (Reprinted by permission, see Fig. 3.)

appear in the blood stream at about the time when bacteremia becomes constant. In both these infections there is a subsequent increasing leukocytosis. This transient granulocytopenia seen following injection of staphylococci or pneumococci differs sharply from the prolonged leukopenia which follows injections of gram-negative bacilli like *E. coli*[10].

By sampling the in-flow and out-flow blood of various organs it can be shown that this sharp decline in circulating leukocytes is produced by their trapping in the pulmonary vascular bed. Ten to 20 minutes later they appear to reemerge from this site[9].

Serial histologic preparations suggest that these leukocytes transiently contained in lung capillaries avidly ingest staphylococci. As leukocytes reappear in the circulation it can be demonstrated that the majority of staphylococci in the circulating blood are intracellular and that such intraleukocytic transport is associated with the failure of the liver and the spleen to remove circulating staphylococci (Figure 5).

That the intracellular site of staphylococci may be responsible for the failure of the reticuloendothelial system to clear them from the circulation is further supported by the fact that animals rendered agranulocytic tend to remove more staphylococci from their circulating blood than do animals with normal leukocyte counts. Furthermore, if staphylococci are placed within rabbit leukocytes *in vitro* and these phagocytes containing bacteria are reinjected into the circulation, such

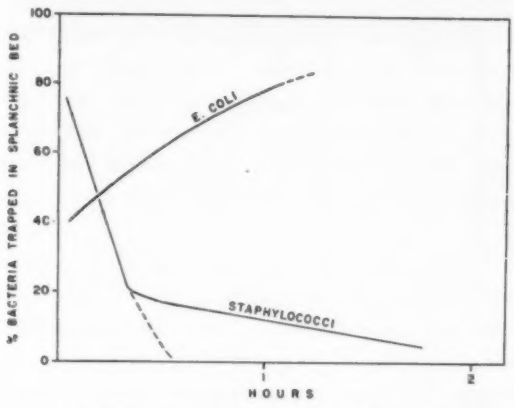

Fig. 5—The characteristics of splanchnic bed trapping of staphylococci and *E. coli* following their intravenous injection.

Splanchnic trapping of staphylococci rapidly declines and generally ceases 20 to 30 minutes after injection of bacteria. In contrast splanchnic sequestration of *E. coli* is unaltered or improves during the course of bacteremia. (Reprinted by permission, see Fig. 3.)

intracellular bacteria appear to remain in the blood stream for longer periods of time than do unphagocytosed staphylococci[8].

Thus the ability of staphylococci to survive phagocytosis may have bearing on the nature and persistence of staphylococcal infection. It may protect staphylococci from antimicrobial drugs present in extracellular fluids. It may allow microorganisms in the blood stream to escape removal within the liver and the spleen where circulating bacteria are usually trapped. It is possible, though unproven, that cells containing staphylococci may lodge in other organ sites and that the phagocytic cell may serve as a focus in which initial multiplication can take place with subsequent focal abscess formation.

The organ selectivity of staphylococci. When injected into the blood stream of experimental animals, staphylococci have a very predictable and intriguing organ selectivity. Although the bulk of microorganisms is rapidly sequestered within the liver and spleen, subsequent multiplication occurs primarily within the tissue of the kidney. Thus the paradox shown in Figure 6 takes place. While staphylococci slowly disappear from the livers and the spleens of infected animals, they multiply rapidly to high titers within the kidneys of the same

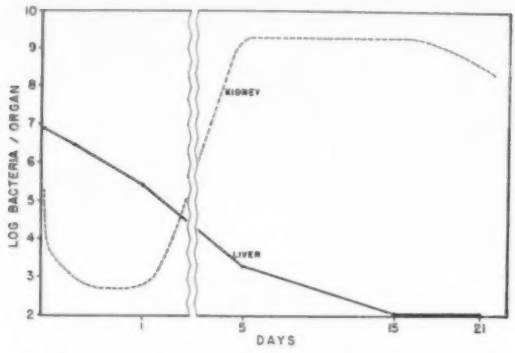

Fig. 6—The behavior of staphylococci in mouse livers and kidneys following intravenous injection of bacteria. Liver titers are initially high, suggesting sequestration in reticuloendothelial cells. Titers fall gradually over 21 days. In contrast, kidney titers rise rapidly after the first 24 hours and remain constant as progressive renal abscess formation takes place. (Reprinted by permission, see Fig. 3.)

animals, resulting in progressive renal abscess formation. The ability to produce progressive renal involvement appears to determine the outcome of the infection.

Why are there such striking differences in the host management of staphylococci which lodge in different organs? This is currently a fascinating unanswered question. It would appear that these differences are probably associated with differences in living cellular processes within the organ rather than limiting growth substances or inhibitors present in certain tissues. For example, when livers and kidneys are removed from the animal body and cell suspensions prepared, staphylococci grow promptly to high titers in preparations of both tissues. These differences in organ susceptibility may offer important clues as to just what biochemical conditions constitute local susceptibility or encourage vigorous staphylococcal multiplication within the host.

The persistence of staphylococci within animal tissues. Most acute bacterial infections either kill the experimental animal in short periods of time or disappear rapidly from organs and tissues. In contrast, staphylococci persist at detectable levels for long periods of time in animals which survive their initial infection. Indeed, staphylococcal infections more closely resemble tuberculous infections than other coccal

disease in the experimental animal. Staphylococci can be demonstrated within the renal parenchyma of infected mice for many months. Bacteremia can be manifest in rabbits for weeks following infection, despite apparent good health. Thus experimental staphylococcal infections are often less decisive than acute bacterial infections, and both the animal host and the microorganism may survive together for prolonged periods of time.

This persistence of staphylococci in host tissues appears to represent an ever present threat to animals with experimental staphylococcal infections. Schaedler and Dubos[11] have shown that non-specific stimuli such as the administration of products of mycobacteria or endotoxin can cause prompt and explosive multiplication of small numbers of staphylococci chronically residing in host tissues to produce progressive disease and death.

It seems probable that this experimental finding has its human counterpart. Job's biblical difficulties with recurrent boils appeared under stressful circumstances. Relapses in chronic staphylococcal osteomyelitis may follow local stress. Patients with apparently healed staphylococcal pulmonary infections may suddenly develop pulmonary abscesses following a non-specific respiratory illness.

The factors which set the stage for vigorous multiplication of staphylococci residing chronically and comfortably within host tissues are completely unknown and have been difficult to approach experimentally. Nevertheless, work in this area offers possibilities for future control of certain problems in staphylococcal disease.

The staphylococcal abscess and staphylococcal infection. Certain facts suggest that the hallmark of staphylococcal disease, the focal abscess lesion, may of itself play an important role in the nature of staphylococcal infection. It has been demonstrated that enormous populations of staphylococci, from 10 million to one billion microorganisms, reside within any abscess lesion[12]. Clinical and experimental experience indicates that mircoorganisms residing in abscesses are not destroyed by antibiotic drugs regardless of their *in vitro* sensitivity[12-14]. It is clear that this is not a problem of inadequate drug penetration into the abscess cavity. Some years ago Robert McCune[12] of our institution showed rather beautifully that penicillin and streptomycin appear promptly within the interior of avascular fibrous wall cavities which mimic the typical staphylococcal lesion. Our own *in vitro* studies suggest that

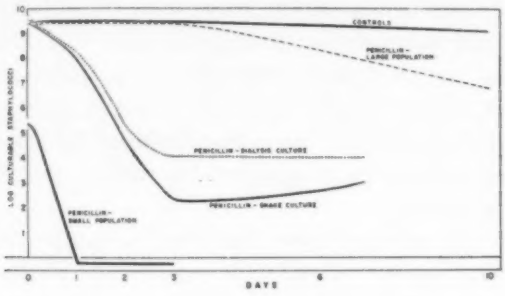

Fig. 7—The effect of penicillin on large and small staphylococcal populations. Despite rapid killing of small populations of staphylococci, large amounts of penicillin do not significantly affect large populations of the same strain unless the microorganisms are stimulated by shaking or suspension in large amounts of media (see text).

populations of staphylococci at the levels found in abscess cavities may be insusceptible because of the crowding and sluggish metabolic activity. When large populations of penicillin-sensitive staphylococci similar to the numbers found in abscesses are exposed to penicillin in test tubes, little or no killing of the microorganisms occurs, despite the fact that smaller microbial populations of the same staphylococcal strain are rapidly destroyed (Figure 7).

If, however, similar large staphylococcal populations are placed on a shaker or enclosed in a dialysis bag suspended in large amounts of media—both situations in which some growth of large numbers of bacteria can occur—the addition of penicillin results in significant killing not seen in ordinary stationary cultures.

These *in vitro* studies appear to have application in experimental infections. The numbers of staphylococci present within certain tissues at the time of antibiotic treatment appear to make a profound difference in the outcome. McCune[14] and Louria[15] have shown that if penicillin or streptomycin treatment is initiated when kidney titers of staphylococci are low, killing of microorganisms and eradication of kidney populations can be demonstrated. If, however, treatment is withheld one to two days, the numbers of staphylococci in kidneys have reached one million or more microorganisms per cc. of tissue. If treatment is started at this point, prolonged therapy results in only a sluggish re-

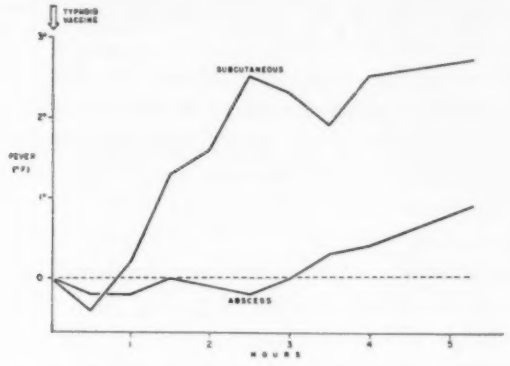

Fig. 8—The effect of the abscess on the febrile response to typhoid vaccine. An amount of subcutaneous vaccine which produces prompt fever in control animals has little effect when given into an experimental subcutaneous abscess.

duction in the number of bacteria and staphylococci persist at high concentrations for many weeks.

Thus staphylococci growing in abscesses are physiologically insusceptible to antibiotics present in the tissues regardless of their *in vitro* susceptibility to the drugs in use, and the sluggish nature of such crowded populations may contribute to this problem.

The abscess and immunity. Staphylococcal infections fail to behave according to the rules of classic immunologic dogma. Antistaphylococcal antibodies are often not demonstrable during infection and humoral immunity is believed of little importance in recovery from staphylococcal disease. These puzzling immunologic peculiarities need re-examination.

We have recently designed studies to test the possibility that the focal abscess does not represent a good antigenic stimulus. This thesis derives from two sets of facts: First, patients with chronic focal staphylococcal disease often fail to develop appropriate antibody to their infecting microorganism, but a prompt rise in antibody is noted when such infections disseminate[16-18]. Secondly, Hughes[19] has demonstrated that fibrin membranes similar to the membrane surrounding the early abscess may operate as a selective filter which will pass only certain proteins and other substances. Thus it appears possible that important

antigenic products fail to get to systemic antibody forming centers from focal staphylococcal lesions.

Although our studies are in their infancy, we already have definite evidence that certain substances placed within an experimentally produced sterile abscess-like cavity do not pass through the abscess wall. For example, the injection of Evans blue or trypan blue dye into such abscess cavities does not result in staining of the surrounding tissues. Further, injections of typhoid vaccine which give prompt and typical fever in simultaneously injected control animals produce little or no fever when placed in the abscess, suggesting that endotoxin transfer across the abscess wall is significantly modified (Figure 8).

Thus we have experimental evidence to suggest that the focalizing nature of staphylococcal infection may operate in multiple ways. The abscess lesion surely plays an important role in the chronic relapsing nature of staphylococcal disease. The poor response of staphylococcal infection to appropriate antimicrobial drugs may be conditioned by the physiologic insusceptibility of large populations of staphylococci residing in abscesses. Indirect and tentative evidence suggests that perhaps the abscess *per se* may influence the immune response to staphylococcal disease by preventing antigenic substances from reaching antibody production centers.

How do these studies relate to the problem of human staphylococcal infection? Extrapolation from animal work to human disease is always dangerous. This is perhaps particularly true of staphylococcal animal research, for to date no experimental animal has been found susceptible to infection by the small numbers of staphylococci believed to initiate human disease. As with poliomyelitis virus, it seems increasingly clear that certain humans represent the most susceptible hosts for staphylococcal infection, and experimental animal systems are gross and cannot be utilized to study many questions of pressing importance.

Despite these deficiencies, certain experimental findings allow tentative explanation of some features of human staphylococcal infection.

First, the work in animals reinforces the clinical impression that major alterations in local or systemic resistance are necessary before staphylococci can invade and actively multiply within human tissues. Although information in human disease is empiric, our own experience indicates that life-threatening staphylococcal infections arise in patients under rather predictable circumstances which affect resistance to all

TABLE I—INTRAHOSPITAL SITUATIONS IN WHICH STAPHYLOCOCCAL INFECTIONS COMMONLY ARISE

1. Patients with probable systemic alterations in resistance.
   - A. Newborn infants.
   - B. Patients with leukemia, Hodgkins disease, lupus erythematosis, diabetes, renal failure, chronic pulmonary disease.
2. Patients with local alterations in resistance.
   - A. Surgical procedures—major and minor (including tracheotomy, urinary catheters, venous cutdowns, multiple irritating injections.)
   - B. Influenza, exfoliative dermatitis, burns.
3. Patients receiving certain types of therapy. (ACTH, steroids, nitrogen mustards, purine or folic acid antagonists, possibly broad-spectrum antibiotics.)

types of infection. At The New York Hospital, over 95 per cent of serious progressive staphylococcal infections have arisen in clinical situations summarized in Table I. Procedures designed to minimize contact with potentially dangerous strains of hospital staphylococci are now an important aspect of the care of these patients.

Secondly, animal studies have shown that staphylococci survive and persist within tissues for prolonged periods. As in tuberculosis, it is clear that ill-defined stresses applied to the host can allow inapparent infections to flare to overt active disease. Staphylococci survive within phagocytic cells and persist in abscess lesions. These characteristics help explain our clinical difficulties in controlling infection with antimicrobial drugs and suggest that therapy should be intensive, prolonged, and combined with surgical drainage of local foci whenever possible.

Lastly, it is apparent that the immunologic peculiarities of staphylococcal infection need more investigation. Although current evidence suggests that classic immune mechanisms do not play an effective role in controlling human infection, perhaps we have not yet asked the crucial immunologic questions. That resistance to staphylococcal disease increases with age is clinically well documented. If it could be shown that immune mechanisms play a role in this process, perhaps we could design better means for preventing disease in certain groups now known to be susceptible to staphylococcal infection.

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EPIDEMIC INFLUENZA*

Panel Discussion

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MODERATOR HARRY M. ROSE: We thought that the topic could best be presented this evening by having three or four of the panel members first present short introductory discussions. We shall then have a period for general discussion and questions, trying to answer written questions as they are submitted from the floor and reserving some right to ask questions ourselves of the panel members.

I should like first to ask Dr. Hirst to speak about the background of epidemic influenza as it has currently been seen in our community, and to say a few words about the responsible strains of virus.

DR. GEORGE K. HIRST: These will probably be the most obvious remarks of the evening, since this is the most publicized epidemic that we have ever had and anyone who has read the newspapers at all will know pretty much what to expect.

The modern history of influenza dates from 1933 which was the first time the virus was isolated in human beings. There are roughly three divisions of our experience with influenza A epidemics. The first of these ran from 1933 to 1947. During that time there were a number of epidemics of influenza A occurring roughly at intervals of two years and the viruses responsible for these epidemics were similar, just how similar we don't know because we did not have good methods of handling and comparing these strains at that time.

In 1943 a vaccine was prepared against several of these strains which was effective in the epidemic of that year. In 1947 this type A strain, which had been widely prevalent, widely seeded in the population and had caused repeated epidemics, disappeared and in this country was replaced rather suddenly in the summer of 1947 by a new strain which was very distantly related and was called A1. The name has no special significance but we did see at that time a new type of episode in the history of epidemic influenza in that a new, relatively unrelated type of strain could crop up, one with which the population had had little or no experience. This type of strain then, was prevalent for the next ten years, changing a little from year to year, causing epidemics of moderate severity, sometimes of world-wide spread. The predictability of the spread was not very high. Those who were students of the situation never knew from year to year exactly when a new outbreak might occur. Last spring, when a note appeared in *The New York Times* that there was an epidemic of influenza in the Far East, the strain was obtained through Army facilities. From the cases studied it was found

that we were entering the third phase of influenza A epidemiology. The antigenic type of this strain, while it was an A strain, was so distantly related to all other A strains as to make it difficult to determine whether it actually was an A strain. Very few people in the general population had antibodies against it. This promised, therefore, to be a repetition of the 1947 experience with a world-wide spread of the disease because the population had no antibodies against the new strain. You have all seen how it spread,—rapidly first in the Far East, Russia, and then in our hemisphere south of us. During the summer months there was liberal seeding of this country without any definite outbreak and then finally blossoming into a full blown epidemic shortly after the schools opened here in the fall.

Throughout this epidemic the virus has been uniform, as far as we know. Definitive studies on this have yet to be done but all strains isolated so far appear to be the same and are very different from any which had previously been isolated. In the laboratory they displayed no particularly distinctive behavior. The main concern, I should say, lay in the fact that the population had had no experience with the strains of this variety.

MODERATOR ROSE: I shall now call upon Dr. Greenberg to tell us how the disease has affected our metropolitan community so far this year (1957).

As Dr. Hirst has already pointed out to you, the strains of virus which are responsible for currently prevalent influenza seem to be closely related to one another. They are causing disease in a population which is largely devoid of antibodies and therefore relatively susceptible. It is not surprising that under these circumstances the disease has assumed epidemic proportions in this area. One of the astonishing things, indeed, is that these proportions were not even larger.

Perhaps Dr. Greenberg can shed some light on this point.

DR. MORRIS GREENBERG: The epidemic of the present type of influenza started in China, apparently at the beginning of the year. As Dr. Hirst told you, a strain of the virus was obtained here around April and the tests confirmed the information from the Far East that it was a new strain of influenza A virus.

In New York City our acquaintance with influenza caused by the new strain began in August, when a number of exchange students arrived from abroad by airplane and by boat. They came from various

parts of Europe and some from Asia and were sponsored by families in this country. There were about 50 students who were quite ill in one of the first boats that came here. About 200 of them had been ill en route, but had recovered. As other boats came from Europe, they began to bring in occasional cases, and we felt certain that the population was being seeded fairly heavily. We did not get any clinical cases in our indigenous population during August, nor during September. A few cases of respiratory disease, suspected of being influenza, did occur. Actually, we recovered the virus from only three individuals who had been living in New York City. Then suddenly, in the last weekend in September, the epidemic of influenza hit the city. We were conscious of it because the municipal hospitals received so many calls for ambulances that they were unable to keep up with the telephone calls and we had to send some to sanitarians to help them out.

(Slide) We tried several ways to determine how intensive the epidemic was. This obviously was not too easy. One could not depend entirely upon reports from physicians because the clinical diagnosis of influenza is not easily made. One thing we did try to do was to find the rate of absenteeism in the schools. This is shown in the lower curve which is rather sharp because we had only 3 points of reference. On October 4th, absenteeism in the schools was about 100,000-150,000. Normally there is a daily absenteeism of between 60,000 and 80,000 individuals. By October 7th, there were some 300,000 absent, a third of the school population. Then the curve began to decline and by the end of October, the number of absentees had fallen to about 150,000. It has since reached normal. The second curve shows the number of cases of influenza reported by physicians. The number kept on rising until about the middle of October when there were approximately 300 cases reported per day. After that there was a gradual decline. This curve is only for the month of October. By now the curve is way down. At present about 25 cases a day are being reported. The top curve indicates the cases of upper respiratory infection that were reported from the municipal hospitals. These were cases that walked into the emergency rooms, cases that were brought in by ambulance, cases that were in the hospitals and cases among hospital employees. Here, too, the peak was reached about the second week in October, about 5,000, and since then the numbers have been gradually coming down. At the present time they are fairly normal.

(Slide) It is interesting that most of the cases occurred in children, children in the elementary and high schools. After that, influenza attacked others in the families of those children. To find out what the situation was among adults, we canvassed a number of industrial concerns. You notice that absenteeism in industry was never very great. Here is R. H. Macy & Co. The peak of the curve was reached about two weeks after the children had their peak and about 12 per cent were absent. The normal rate of absenteeism is about 5 per cent. The Metropolitan Life Insurance Company had a similar peak, about 12 per cent absent as against a normal rate of about 5 per cent. Consolidated Edison Company never had more than about 7 or 8 per cent absenteeism and that also held true for the New York Telephone Company and for the New York Transit Authority. There were very few absences among policemen or firemen and there were few absences among the personnel of our own Health Department.

(Slide) This curve is taken from Collins' paper which shows that we have had many epidemics of influenza in this country, as Dr. Hirst has said. We have had some 21 epidemics of influenza since 1918, which is that first curve. These were measured by the excess deaths from influenza and pneumonia. It is a very good measure. It is much easier to measure deaths than it is to gauge the number of people who are sick. Note the many peaks in 1921 and 1922 and so on. Notice that these peaks gradually get lower and lower, demonstrating that the more recent epidemics were not as severe as the earlier ones.

(Slide) In this city we also measure the mortality from pneumonia. The thin, solid line is the normal curve, the expected trend. Notice that in the epidemic of 1953 there was an excess number of deaths from pneumonia. The big curve represents the excess number of deaths from pneumonia this year, beginning toward the end of September and continuing into mid-October. By now that curve has come way down.

(Slide) You can also measure the extent of an epidemic by total mortality. Here is the expected curve, the solid line; the broken line represents the actual death rates and the other line is the infant death rate. This is an ordinary year, not an epidemic year, and the three curves are close to each other.

(Slide) In 1943-1944 when we had an epidemic year, you notice there was a rise in total mortality. The infant deaths, however, did not rise. They remained within the normal range. I stress that because we

had been told at the beginning of the epidemic that most deaths occur in very young infants and in old people. That isn't so. They occur in old people but not in very young infants.

(Slide) Here is the curve for this year and you notice that the total mortality went way up and that this is true also for old people over 65. But the infant mortality stayed within the hatched area, which is within the expected limits. These curves, by the way, have come down markedly at this date (November 7th).

(Slide) During the month of October we had 151 deaths associated with influenza and the majority of those were in the old age group. Those over 50 years of age accounted for 51 per cent of the deaths. There were comparatively few in those under 10 years of age. We had no death in a child under one year in the month of October. If you separate male and female deaths, the death rate in the older group is much higher in males than in females. That was largely due to the fact that most of these deaths occurred in people with chronic heart disease, atherosclerotic heart disease and coronary thrombosis, diseases which occur more frequently in males than females. On the other hand, in the age group 20-29, deaths in females made up 29.5 per cent of the total, while in males they accounted for only 6 per cent. This was due to excess deaths associated with pregnancy. In pregnant women there were two and one-third to two and one-half times as many deaths in the month of October this year (1957) as last year.

If the death certificates are examined and the secondary or associated conditions are classified, it is found that arteriosclerotic heart disease was present in about 33 per cent of the cases, pneumonia in 31 per cent, pregnancy in 10 per cent and 6.5 per cent had rheumatic heart disease. This last group is actually larger because there were three deaths from rheumatic heart disease in pregnant women which were not included in the rheumatic heart disease group. About 5 per cent each had cancer and diabetes, about 3 per cent asthma, and other miscellaneous conditions made up the rest.

In August we ordered some vaccine with the expectation that it would be given to city employees engaged in essential city services. However, we received only a limited supply of vaccine. By the end of September some of our supply was turned over to the Department of Hospitals to immunize doctors and nurses and then we began to immunize firemen and policemen. The bulk of vaccine was not received

until the end of October when the epidemic was nearly over.

During the height of the epidemic when the municipal hospitals were being overwhelmed with patients, we opened several of our District Health Centers to take the overflow. These centers were open day and night, Saturdays, Sundays and holidays, and patients were treated there.

Conferences with the Department of Hospitals were held daily. Because the ambulances could not take care of all the cases, arrangements were made for interns to go out in police cars and see patients in their homes and treat them there; only those patients with pneumonia or other complications were removed to hospitals.

We also informed all the doctors and hospitals in the city that our laboratory was equipped and able to process any specimens sent to us. We received quite a number. We received a great many specimens of lungs obtained at postmortem. From 25 of these, influenza virus was recovered. Incidentally, from 20 of those 25 we also recovered the staphylococcus in fairly concentrated culture. We also tried, to the best of our ability, to keep the public informed of what we knew. It is quite true that we did not know a great deal about this new strain. In fact, nobody knew very much about it because it was new and very little work had been done with it. However, we did have some information about the natural history of this type of influenza which we shared with the public.

I shall not go into a discussion of vaccination nor of pathology nor treatment since these matters will be discussed by other members of the panel.

MODERATOR ROSE: Would you care to hazard a guess as to why the epidemic, which was proceeding merrily on its way in the early part of October, suddenly began to decline?

DR. GREENBERG: Reports indicate that in the Far East, in the very thickly populated areas, the incidence was about 20 per cent, over-all. In some areas, of course, in small groups, incidence rose as high as 50-60 or even 80 per cent, but on an over-all basis it was about 20 per cent. On the other hand, in Australia where the population is less concentrated, the percentage was about 10 per cent. I judge that in this city it was about 5 to 8 per cent. It is difficult to give an exact figure.

It is also interesting that the mortality, which was about 0.1 to 0.2 per cent in the Far East, was only about 0.02 to 0.03 per cent here; about 0.1 of what it was in the Far East.

MODERATOR ROSE: In other words, Dr. Greenberg, you feel that the decline in incidence of the disease does not actually reflect the fact that we are approaching saturation so far as the population of the community is concerned?

DR. GREENBERG: I am sure that a great part of the population has been infected without showing symptoms but I have no doubt that there is a fair percentage of the population which has not been infected. That of course bears on the question whether we should continue the vaccination.

MODERATOR ROSE: That is why I asked the question and that is the answer I wanted to get from you. We shall come back to it a little later.

I shall now ask Dr. Helpern to discuss some of the findings in the fatal cases of influenza.

One of the considerations that has interested a great many of us is the mechanism which actually causes death. It has been known for many years that animals to which influenza virus has become adapted may die as the result of the virus infection alone. On the other hand, it has been generally believed in the majority of fatal cases in man that superinfection with some bacterial agent, generally staphylococcus, has been responsible for death, at least in the cases that have been seen during the last 20 years or so. On this account it has usually been said that we have effective means, in the form of antibiotics, which will enable us to control secondary bacterial infections and to prevent death in the majority of instances. However, I think I can assure the audience that there are situations when influenza occurs without superinfection, in which we are currently quite helpless. Dr. Helpern, would you comment on that point?

DR. MILTON HELPERN: Yes. The Medical Examiner's Office is involved in this particular study because all sudden and unexpected and medically unattended deaths in New York City are referred to this agency for investigation. When such deaths occur in young people it has been the policy of the Medical Examiner's Office to perform an autopsy as part of the postmortem examination to determine the cause of death. Such a situation presented itself this year in which deaths from epidemic influenza were encountered. The fatalities from this cause began to appear sporadically about the middle of August and then became increasingly more frequent toward the end of September and continued all through the month of October, our incidence cor-

responding very closely to that which Dr. Greenberg has already given. An unusual number of unattended deaths in the home were reported to the Medical Examiner's Office of persons who had been in apparent health and concerning whom a history was obtained of a fairly rapid onset and progression of a respiratory illness which, initially, did not suggest anything serious enough to warrant calling a physician.

I might say that from year to year the Medical Examiner's Office has had an occasional similar case referred to it.

Rare deaths from influenza and its complications have been encountered by the Medical Examiner in non-epidemic years and have been verified only by the means at hand, chiefly the pathological appearance of the lungs. Prior to this year we did not have the opportunity of checking the pathological findings with viral studies and in this connection I should like to acknowledge the great help we received from the New York City Health Department Virus Laboratory by their determination of the presence of influenza virus in most of the cases that we encountered and in which the lungs were submitted to them for viral studies. The check was a very close one indeed.

The first case brought to our attention occurred in the middle of August and was that of a 17 year old boy, one of a group of exchange students who came over on the steamship Arosa Sky. He was one of many others who had been mildly ill with influenza on the boat but his symptoms suddenly became worse after debarkation. He was hospitalized promptly but developed very severe toxic symptoms with fever and died about 24 hours after admission to the hospital. The death was reported to the Medical Examiner's Office and an autopsy was performed by Dr. Vance, Deputy Chief Medical Examiner, who had autopsied a good many of these cases during the 1918 influenza epidemic, and other similar cases occasionally from year to year. His experience led him to diagnose this as a death from influenzal bronchopneumonia. Subsequent studies by the Health Department revealed the presence of the Asian strain of influenza virus which was the etiological infective agent in all the cases here reported.

Our next case did not occur until the latter part of September and from then on, and through the month of October, we had a fair number of them. I should like to point out that in our series of cases the subjects were not debilitated. They did not have other diseases and most of them were adolescents and young adults. In all the cases, there was gross

and microscopic evidence of an influenzal type of bronchopneumonia. In this connection, I should like to point out that the Medical Examiner's Office encounters many cases of death from unsuspected lobar pneumonia. I think we see more lobar pneumonia in our autopsy material than do the hospitals. Lobar pneumonia is now rarely fatal in hospital practice but it is not uncommonly encountered as an unsuspected cause of natural death in the Medical Examiner's material. In recent years it has been the second most common cause of sudden natural death disclosed by our autopsies, ranking second only to occlusive coronary artery disease. Lobar pneumonia is readily distinguished at autopsy from influenzal pneumonia, which is the subject of tonight's discussion.

(Slide) This represents the incidence of influenzal deaths in Manhattan and Brooklyn autopsied by the Medical Examiner's Office. Most of these occurred during October, only one in September and one in August. The ages were 17, 17, 14, 9, 13, 12, 30, 6½, 42, 41, 22, 35, 37, 23, 46; there were 10 Negro and 5 white persons, 10 females and 5 males. The influenza virus was cultured from most of the cases. The case of the 14 year old boy in Brooklyn was very interesting. The virus was recovered from the lungs on culture. This boy had a respiratory infection and the family, because of their knowledge of what they had read in the newspapers, that the disease was epidemic, correctly assumed that the boy had influenza. While at home in the care of a relative who was old and somewhat helpless, he went berserk and leaped from the window and died of his injuries; the autopsy revealed that death had resulted from injuries but that he also had an influenzal type of bronchopneumonia from which influenza virus was recovered.

One of the cases is that of an eight months pregnant, 20 year old Negro woman who had a fulminating infection with fairly characteristic findings in the lungs from which the virus was recovered. Out of curiosity the placenta was also submitted for culture. However, Dr. Widlock, who carried out the viral studies, reported that there was no virus in the placenta.

Four of the cases, two adults from Brooklyn and two from Manhattan, revealed pathological findings recognizable as those of influenzal pneumonia. Material from these cases was not submitted for culture. As for the incidence of fatal cases investigated by this office, in the other boroughs, there were five cases in Queens, two in the Bronx and one in Richmond. So that in all there were 23 cases of fairly rapid acute fatal

illness referred to the Medical Examiner's Office during the short period of time mentioned, in which death was primarily the result of influenzal bronchopneumonia.

I should like to show illustrations of the pulmonary lesions which I think are very typical of the disease and yet, while it is the most strikingly severe case in our present experience, it does not quite match in severity the lesions that were encountered and described in the 1918 epidemic in which the lungs revealed the very remarkable and bizarre changes described and illustrated in *The Pathology of Influenza.**

The case I am now illustrating has the most severe pulmonary lesions in this series and most of the others have comparable findings. (Slide) This is a colored photograph of the lungs of a 13 year old girl, who was ill at home for about 24 hours in all. She came down abruptly with a respiratory infection with fever and cough. Within a few hours she began to expectorate blood, became cyanotic, went into shock and collapsed and died without medical attention. The lungs in this case are quite characteristic. There are large areas of confluent hemorrhagic bronchopneumonia of the right upper and lower lobes with similar large elevated hemorrhagic areas and a dull red discoloration of the pleura of the left lower lobe. (Slide) Another photograph reveals similar changes on the posterior surfaces of the lungs. The mucosa of the trachea and bronchi appears swollen, intensely congested and in places hemorrhagic and covered with a gray pseudomembrane, the mucosa having a necrotic appearance in places. The primary lesion of this disease is this acute necrotizing pseudomembranous tracheobronchitis and the inflammation spreads into the parenchyma and interstitium of the lung. The smaller bronchi and bronchioles are surrounded by gross and microscopic zones of bronchopneumonia with larger areas of confluent hemorrhagic bronchopneumonia. Intrabronchial plugging by secretion and exudate occurs and a conspicuous feature is the edema and hemorrhage of the parenchyma. Subpleural collections of serosanguinous exudate are found in the inflamed bronchi. A photomicrograph of a large bronchus reveals necrosis and sloughing of the mucosa. The partly sloughed off necrotic mucosa is covered with fibrin enmeshed with red blood cells and some leukocytes. Dilated mucosal vessels contain fibrin thrombi. There is considerable edema, congestion and some interstitial hemorrhage. Other sections of lung reveal incompletely consolidated

* Winternitz, Wason and McNamara, Yale University Press, 1920.

parenchyma with over-distention of some of the alveoli. There are patchy areas of acute bronchiolitis and bronchopneumonia. In some sections, many colonies of bacteria, probably staphylococci, were found in the involved parenchyma. Considerable edema and not very much leukocytic exudate although some, both mononuclear and polymorphonuclear leukocytic in type, were seen. An area of the same lung under slightly higher magnification reveals a small bronchiole with its mucosa sloughed off and very intense inflammation of the surrounding parenchyma in which there are colonies of bacteria representing secondary invaders with resulting distortion and over-distention and rupture of air vesicles. The alveolar and bronchiolar effect is partly inflammatory and partly mechanical as the result of the obstruction of the smaller bronchi. Other sections of the same lung reveal considerable interstitial inflammatory edema, and areas of hemorrhagic bronchopneumonia.

The pneumonic areas are almost solid and filled with blood which is partly hemolyzed and confluent due to rupture of interalveolar septa, with considerable over-all damage to the lung. Small areas of hemorrhagic pneumonia surround inflamed bronchioles. In some areas of hemorrhagic pneumonia, colonies of bacteria, representing secondary invaders, are conspicuous and the blood here is partly hemolyzed.

In another case, that of the 20 year old pregnant woman, that was not cultured for the presence of virus, the necrotizing bronchiolitis is conspicuous. In this section the inflammatory exudate lies partly on the mucosa and penetrates and partly destroys it, infiltrating the interstitium. Sections of the first case reveal very characteristic intense lesions in the lining and wall of a fairly large bronchus.

(Slide) The sections reveal necrosis of the bronchial epithelium, mononuclear and slight polymorphonuclear leukocytic infiltration and infection with secondary bacterial invaders. The pathological lesions in all the cases studied are those of influenzal tracheobronchitis and bronchopneumonia.

MODERATOR ROSE: Dr. Rogers and Dr. Kilbourne have been much interested in these cases of fatal influenzal pneumonia and have studied them bacteriologically and for virus, as well as noting the histologic changes. I should like to ask Dr. Rogers to comment briefly on the findings.

DR. DAVID E. ROGERS: It might be helpful for me to outline certain

clinical syndromes that we have seen arising during the current influenza epidemic.

When the epidemic first reached New York it was decided that we would admit to the hospital only patients who had lower pulmonary tract complications of influenza. Consequently my remarks are confined to this very small segment of the population who were admitted with pneumonia secondary to influenza. We have become impressed with two very different syndrome groups.

One group of patients have had clinical illnesses which have resembled those that Dr. Helpern has just discussed. These individuals have developed influenza, followed by a period of declining symptomatology. They have then become worse precipitously and have been admitted with symptoms that we associate with any type of bacterial pneumonia. These patients have had pleural pain, typical signs of *local* consolidation, and sputa which reveal many bacteria on smear. They have had x-ray changes compatible with local bacterial consolidation and they have responded rather promptly to antibacterial therapy. The majority of these pneumonias have been caused by pneumococci. We have also had cases of secondary staphylococcal pneumonia similar to those Dr. Helpern has discussed, though to date we have not lost any patient with what we believe to be the pure secondary bacterial pneumonias in association with influenza.

We have, on the other hand, seen another group of patients who present quite a different clinical syndrome. These patients, usually rheumatics with a mitral lesion, have uniformly had preceding cardiac disease. They have had typical influenza which has been followed without any remittive period by increasing respiratory distress and cyanosis. On examination these patients have been acutely ill. They have had striking tachypnea and dyspnea. They have had *no* signs of local consolidation. On physical examination, the principal findings have been those of diffusely suppressed breath sounds, rather prominent expiratory wheezing, and as the disease has progressed, fine diffuse rales over all lung fields. Chest x-rays have revealed massive bilateral infiltrates—a picture which has been indistinguishable from that seen in cardiogenic pulmonary edema. Nevertheless these patients have had no signs of right-sided failure. Venous pressures have been normal and no peripheral edema has developed. These patients have had high sustained fevers. We have been unable to culture significant bacteria in their sputa or from

their lungs at autopsy. The clinical course has been characterized by progressive increasing dyspnea, cyanosis and profound oxygen unsaturation. Five of the seven patients with this syndrome have died of their disease.

Perhaps Dr. Kilbourne might indicate his findings in the laboratory on this group. We have found no bacteria on rather carefully titered cultures.

MODERATOR ROSE: Dr. Kilbourne, would you tell us what you have found?

DR. EDWIN D. KILBOURNE: First of all, I would like to indicate that Dr. Rogers is so intimately familiar with his material that he slipped a gear tonight and failed to mention that these patients, the latter group, are indeed distinguishable from patients with pulmonary edema in that they have in addition to the findings of pulmonary edema, bronchial and bronchiolar necrosis which would characterize them as being probably influenzal in origin. Even at the pathological level of our studies there is considerable evidence that they are different from rheumatic heart disease patients who are dying of pulmonary edema. We have been surprised to find how easy it is to isolate virus from these patients. The virus is present in the lung in fairly high concentration. We have thus far titrated two of the lung specimens in detail and this would indicate that it is probable that the virus is just there as a contaminant from tracheal secretions. The titers of virus in the lungs have been appreciably higher than we find with titrations of virus concentrations in the throat washings.

If Dr. Rose will permit, it might be helpful to give some background concerning what we believe to be the importance of observations of this kind which are far from limited to our group. I have heard that similar cases have occurred in Cleveland and Denver. This may prove to be a universal phenomenon, when further cases have been carefully studied.

The people who dealt with the 1918 phenomenon have been debating for years just what caused the deaths in 1918, and I think some of this debate has given rise to the feeling and the predictions that we have seen in the press that the deaths were due mainly to secondary bacterial infection. I am sure that many of them were. There was a great controversy about what sort of secondary bacterial infection caused the deaths, depending upon the part of the country in which one lived.

If one died in an Army camp, he died of streptococcal or pneumococcal, or hemophilus influenzae, bacterial pneumonia.

In addition to these cases there has always been evidence—well reported -which would indicate that some of the deaths were so extremely rapid that it was doubtful that they could be explained solely on the basis of secondary bacterial infection.

I think that with the modern virus isolation techniques now available in this epidemic it is interesting to find some verification of the fact that people can die of influenza virus infection alone. This is somewhat terrifying because it is clearly indicative of a situation in which we cannot anticipate assistance from antimicrobial therapy. As Dr. Rogers indicated, the cases we have lost have not been those which have been primarily influenza virus pneumonia.

MODERATOR ROSE: We can certainly confirm in all details what Dr. Kilbourne has said about the etiologic factors and the importance of influenzal pneumonia *per se* as the cause of death. Thus far there have been seven deaths from acute fulminating pneumonia at Presbyterian Hospital which could be ascribed in all likelihood to influenza or influenza aided by secondary bacterial infection. In three of these seven cases it was possible to demonstrate in the lungs large numbers of staphylococci that presumably played a role in the death of the patient. In all seven of these cases it was easily possible to isolate the virus from the lung, so there was no question about the diagnosis; moreover, the histopathologic findings were those that have been described. I would certainly agree that tracheobronchitis is one of the outstanding and most characteristic features.

As I indicated a moment ago, three of these seven cases apparently had complicating staphylococcal infection. In the other four there was no evidence that any bacterial agent contributed to the patient's death, which must be ascribed to pneumonia caused solely by the virus. So, in a certain proportion of the cases we were unable to control events by any of our current therapeutic methods. It is somewhat frightening to think what might happen if the disease recurred and if there were a greater incidence of cases of this sort. Let us hope that nothing of this sort actually happens and that meanwhile we can find a way of dealing adequately with the problem.

I should now like to call on Dr. Fisk, who is here this evening as a substitute for Dr. Kneeland who unfortunately was unable to be with

us because of illness. I wonder, Dr. Fisk, if you will comment on the clinical aspects of the disease as it may be seen by the practicing physician and say what you wish about therapeutic measures.

DR. SHIRLEY C. FISK: As indicated by the other members of the panel, influenza as seen by the practicing physicians here in this city is a mild infection and can generally be treated at home with supportive measures. However, a very small percentage of patients has fulminating pneumonia with secondary invaders which may conceivably play a role in the fatalities. For this reason I think we must be extraordinarily careful in following cases to determine whether they are mild and self-limited or complicated by pneumonic involvement, either of primary or secondary origin.

In the cases seen in hospital, the procedure is relatively simple, at least therapeutically. One uses antibiotics in an attempt to suppress or control the bacterial aspect of the disease and in the instances that we have seen at Presbyterian Hospital the staphylococcus was our major problem. Most of these organisms were susceptible to most antibiotics, unlike the resistant staphylococci seen in hospital infections. It is our belief that adequate results are achieved in these cases only if treatment is initiated before culture sensitivity studies are done and reported. To begin treatment it is best to use the bacteriocidal drugs, penicillin and streptomycin; and, if the sensitivity studies show the staphylococci to be resistant to these, more appropriate broad-spectrum drugs such as chloramphenicol should be used.

Fortunately these cases with pneumonic involvement represent a small minority of the general group. We believed that the cases presenting themselves at the clinic without evidence of pulmonary infection should not be treated with an antibiotic but should be treated conservatively with aspirin and bed rest at home.

MODERATOR ROSE: Thank you, Dr. Fisk.

I think we should now speak about immunization. We shall first present some relevant data and then get on to the questions—there are a lot of them and many are extremely interesting.

Some of the speakers have already covered some of the points which were raised in your questions. When it became apparent, as Dr. Hirst said in his introductory remarks, that we were confronted with a viral agent to which the population at large had no demonstrable antibodies, it seemed only prudent to consider the use of an immunizing agent

which might conceivably induce sufficient protection in the population to reduce at least the severity and incidence of the disease, if not to prevent it altogether.

We had as a precedent the results of studies which were started a number of years ago and have since been continued by the Commission on Influenza and the United States Public Health Service. These indicated that a properly constituted influenza virus vaccine, i.e., a killed vaccine containing enough antigen similar to the strain or strains prevalent in the community, would have at least some immunizing effect.

Very early in the manufacture of the vaccine it became apparent that production of a polyvalent vaccine was virtually impossible. A monovalent vaccine containing one of the prevalent strains was therefore considered. Difficulties were encountered in the manufacture of this product because these strains are somewhat peculiar compared with others so far as the amount of virus produced in the developing hen's egg is concerned, particularly the amount of hemagglutinin. Since hemagglutinin is one of the indexes determining the amount of antigen in vaccine, it was obvious—I won't go into the reasons—that the amount of virus acceptable in the vaccine would have to be somewhat less than the optimum. It was on this account that a vaccine containing 200 chick cell agglutinating units per milliliter was decided upon. More than 40,000,000 milliliters of this vaccine were released by November 9, 1957, of which the majority had been distributed to civilian agencies. This represents a considerable achievement by the manufacturers if one realizes that the vaccine was in short supply until recently. In any event, with the vaccine in production, a number of questions arose: Is it any good? Will it protect against influenza? If so, how much should be given, by what route, how often, etc.?

As mentioned earlier, studies concerning experimental immunization against influenza have been going on for some years under the auspices of the Commission on Influenza. One of these studies has been under way at Fort Dix, another at Lowry Air Force Base and another at Fort Ord. In addition, the Navy has conducted similar work at Great Lakes Naval Training Station, and another study has been done in Wilmington, Delaware, by the Public Health Service. In each of these places an experimental program was laid out during the summer to ascertain as quickly as possible the effectiveness of the vaccine.

At Fort Dix, vaccine was received on July 28. The immunization

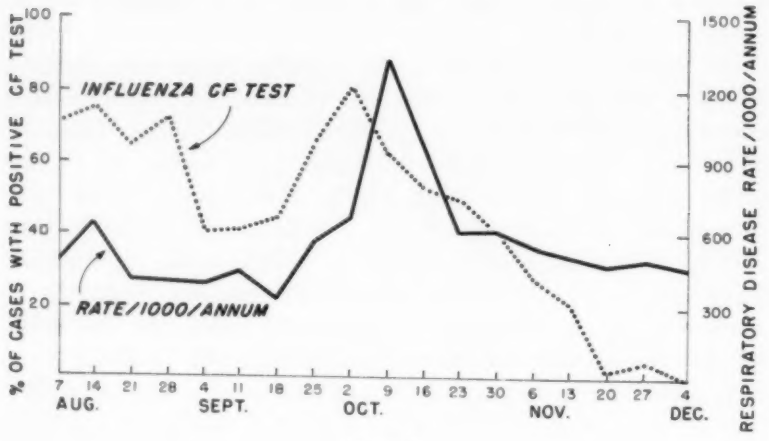

TABLE I

program was begun on August 1, and the supply of vaccine was exhausted by September 6. There were two vaccines available, one of the sort that has been distributed to the general public, containing 200 CCA units per ml., and the other an experimental vaccine containing 750 CCA units per ml. These vaccines were given in single doses of 1.0 ml. subcutaneously to recruits, distributed by companies so that one-third of the men in each company received the 200 CCA unit material, one-third got the 750 material and one-third remained as controls. Altogether, by September 6, there were 1869 men in the 200 CCA unit group and 1665 in the 750 CCA unit group, as you will see in a moment on the slide.

As Dr. Greenberg has indicated, influenza became very prevalent toward the end of September and on the first slide we shall see what the respiratory disease picture was at Fort Dix at that time.

(Slide) (Table 1.) This slide is a graph of the total rate of respiratory disease by hospital admissions. On the vertical axis the hospital admission rate per 1,000 per annum is plotted. On the horizontal axis are date lines for the months of August, September, October and November, up to the week ending December 4. The solid line indicates the hospital admission rate for acute non-bacterial respiratory infections. This, of

TABLE II—EFFECT OF IMMUNIZATION WITH MONOVALENT VACCINES ON HOSPITAL ADMISSION RATES DURING EPIDEMIC PERIOD OF ASIAN TYPE INFLUENZA

Week Ending	*200 CCAU (1869)*	*750 CCAU (1665)*	*Controls (1655)*
2 October	7	5	30
9 October	20	8	36
16 October	22	11	51
23 October	9	7	9
Total	58	31	126
Rate/1000	31.0	18.6	76.1
% Reduction	59	76	—
Ratio	1/2.4	1/4.0	—

course, would include both cases of influenza and cases of respiratory disease caused by other viruses, so it is a crude way of showing the picture with respect to influenza. Back in August it can be seen that for the week ending on the 7th, about 70 per cent of the men who entered the hospital had serologic evidence of influenza. The rate climbed to about 75 per cent by the middle of August but had fallen off considerably by early September. Subsequently you will see that there is an extraordinarily high peak reaching its height on October 9, at which time the rate for influenza during this time was approximately 85 per cent.

(Slide) (Table II.) Let us look at the next slide and see what happened to the study groups. These are the data for the weeks ending October 2nd through October 23rd, during the period when the big peak in incidence occurred. There are three columns indicating the number of cases from each of the groups admitted to the hospital in these periods, for those vaccinated with the 200 unit material, the 750 unit material and the controls; the numbers in parentheses, 1869, 1665 and 1655 are the actual numbers of men in each of these groups. There was a total of 126 cases admitted from the controls, only 31 from the 750 unit group and 58 from the 200 unit group. You can see that the reduction was 59 per cent in those given the 200 unit vaccine and 76 per cent in those given the 750 unit vaccine. The protection ratio was

1:2.4 for the first group and 1:4.0 for the second. These data clearly indicate that the 200 CCA unit vaccine had some protective effect, although it left a good deal to be desired. Also, that a vaccine of greater antigenic potency exerted a better protective effect.

You may be interested to know that the results obtained by Dr. Gordon Meiklejohn at Lowry Air Force Base with the 200 unit vaccine showed a reduction of 60 per cent as compared with our 59 per cent. He used 400 unit CCA vaccine as an alternate, instead of the 750 unit material which we employed. His reduction with the latter vaccine was 75 per cent as compared with our 76 per cent, clearly indicating that the results are probably significant and that the 200 unit vaccine is actually not a completely satisfactory product although it will afford considerable protection.

On the basis of studies of this kind, the Public Health Service recommended that the amount of virus in the vaccine be increased and that the potency be raised from 200 CCA units per ml. to 400 CCA units per ml. The 400 unit vaccine became official on November first and on that date production of the 200 unit vaccine was discontinued. It was recommended that 1.0 ml. of the 400 CCA unit vaccine be given subcutaneously for immunization.

It was also found in the course of these studies that inoculation of 0.1 ml. intradermally in adults is not an adequate immunizing procedure, although this amount of vaccine followed by another similar dose may be effective in children.

Dr. Kilbourne, you have had experience with these matters, would you like to make some comment?

DR. KILBOURNE: Our experience was predicated on the necessity of vaccinating people at New York Hospital and we were aware of the background of a great deal of this military experience and the extensive work with immunizing agents containing influenza virus in the past. However, recognizing that we were using doses which were less than optimal, we were forced, as were, I think, a great many practicing physicians, into the necessity of trying to immunize 100 people instead of 10. There was evidence in the literature to indicate that intradermal injection or perhaps more specifically double injections, or multiple injections of antigen—intradermal or not—would give a far better immunizing stimulus than the same quantity of vaccine in a single injection. Perhaps Dr. Rose will comment on this later, but I think for rea-

sons of the technical details of administration of vaccine and the like, the military has never been enamored of the idea of giving multiple injections or giving it by the intradermal route. So, actually there was not a great deal of practical information on this point. Therefore, we thought that in the course of immunizing our personnel we would acquire information more germane to civilian practice.

(Slide) This represents a small experiment compared with the military experience—with the Fort Dix experience—but it indicated to us that intradermal immunization had some worth. Inadequate as it may be, if you look at the first schedule, which is 1 ml. subcutaneously, it compares with the administration of 1/10 of this amount, twice, in spaced injections one week apart. Most interesting to us is the fact that if you split the 1 ml. and give it in two injections, a week apart, you do considerably better. This particular technique of antibody measurement produces from 14 per cent to 49 per cent demonstrable antibody.

Comparable with this experience and this study of antibody, Dr. Rogers and I observed an epidemic in the hospital involving patients who were not immunized and involving some other personnel who had been immunized. They had been immunized with the two "inadequate" schedules of 200 CCA units in one injection of old vaccine or in some cases with only 1/10 of a milliliter intradermally, and there was definite evidence of protection against clinical disease in both these groups.

MODERATOR ROSE: Dr. Hirst, would you care to discuss the relationship of antibody titers, as determined by hemagglutination-inhibition tests, to protection?

DR. HIRST: I think it is fairly clear now that the antigen in the virus which induces an antibody responsible for protection, is an antigen which is able to make red cells agglutinate. This has been shown by fluorescence antibody technique, not only for influenza but for fowl plague virus, which falls in this same general group. There is a general correlation between hemagglutination antibody and protective antibody as measured in the laboratory and there is the negative correlation for certain other antibodies. As far as field studies go, there have been some fairly good correlations of protection with circulating antibody level. What we don't know is the answer to the question raised here—about the protective level when we encounter a new virus giving antibody responses which on the whole are relatively low compared to those we are accustomed to getting with

other strains. Where the level of protection lies is hard to say. It is possible that these low antibody levels are as protective as the higher levels against other strains.

MODERATOR ROSE: Very good. If we wish to get out of here before morning I presume that we had better get on with the questions. A whole series of questions along similar lines are written on the papers which lie before me. Some of them we can answer, at least in part.

Dr. Fisk, here is one for you: What would be a working clinical method to distinguish between adenovirus infections and Asian influenza?

DR. FISK: Short of viral studies, these cases would be difficult to differentiate clinically. Let us classify them all as respiratory and say they are all self-limited and subside spontaneously without any specific therapy.

MODERATOR ROSE: In other words, you feel that even when influenza is prevalent in the community, the fact that a patient has an acute respiratory infection with some fever does not necessarily mean he has influenza?

DR. FISK: Yes.

MODERATOR ROSE: We arrive then at the unhappy conclusion that the clinician is stuck for an etiologic diagnosis.

DR. ROGERS: I would like to take issue with that.

MODERATOR ROSE: I wish you would.

DR. ROGERS: Dr. Kilbourne and I have been commenting on this recently. I think we agree that one can make a fairly reasonable etiologic guess at the clinical level regarding the specific virus producing a clinical syndrome. Therefore this appears to be a very reasonable question. The vast majority of cases of influenza can be distinguished at a clinical level from the great majority of adenovirus infections or the common cold. Let me give just a short example. In our experience, influenza has been an illness in which the patient goes from relatively good health to fairly striking illness within a short period of time. He is well in the morning, sick in the evening. He has a prominent cough, which is not an early feature of a cold. He has a striking headache; he has very few nasal symptoms. I am oversimplifying this, nevertheless I think you can make a sound case for differentiating viral illnesses at the clinical level and be right 60 to 75 per cent of the time.

DR. FISK: I don't like to argue the point.

DR. ROGERS: Please do.

DR. FISK: I think if you see the cases in the home or if you see them particularly in your own home where you can observe the early prodromata you will be struck by the fact that there is a period of 12 to 24 hours during which the patient will have vague myalgias, slight headache, mild congestion of the nose, slight sore throat before the acute onset, mentioned by Dr. Rogers, of severe headache, fever to 104° F., prostration and cough.

DR. KILBOURNE: That is not a common cold.

DR. FISK: I would at this point agree it is not a common cold. I still think that as a clinical entity it is difficult to distinguish. I think we assume most of these respiratory infections are influenza but in a non-influenza year one would see this same picture reproduced—call it then la grippe.

DR. ROGERS: I feel that la grippe presents quite a different clinical picture than influenza. I would like to debate this further but I think we probably should go on.

MODERATOR ROSE: We can stay here for quite a long time to debate this interesting point, which is of real moment to the practicing physician who is responsible to his patient and has to make up his mind. He has to deliver an important message to the family concerning the diagnosis. I think I would be inclined to agree with you to a certain extent, Dr. Rogers. I have seen so many cases of influenza that were difficult to distinguish, even in a season when influenza was prevalent, that I would not want to stick my neck out and do anything more than bet the percentages. As far as diagnosis is concerned, I think we are approaching the time when the clinician may have more accurate diagnostic methods at his disposal, but I don't know exactly how accurate some of the new techniques are.

Consider, for example, the problem presented when a known agent, such as type A influenza, suddenly becomes prevalent in the community. Realizing that a large proportion of respiratory disease may be caused by this virus, it is possible, by means of recent techniques employing tagged antibodies, to investigate nasal smears taken from the patient and to see whether specific antigens can be demonstrated by flooding the smear with tagged antibody, washing and examining it with the fluorescence microscope. This is not the sort of equipment that every practicing physician has in his office, but it is certainly available on a fairly

wide scale in health centers, teaching institutions, and commercial laboratories. This technique is actually under investigation at the present time. Perhaps Dr. Hirst is in a position to say something about it.

DR. HIRST: As a matter of fact, I was going to look up the data on the fluorescence technique for virus in sputum. It can be done if you have the reagents and it permits a quick bedside etiologic diagnosis.

MODERATOR ROSE: In any event, it is along these lines, I suppose, that the physician will be given methods which eventually will eliminate some of the uncertainty that he now experiences when confronted by problems of this sort.

Kindly unwrap your crystal ball, Dr. Greenberg. Here is a question for you: Is the second wave of influenza in Japan caused by the same strain of virus and do you think that there is going to be a second outbreak of influenza in the United States?

DR. GREENBERG: I suppose that the questioner means, are we going to have a second very severe outbreak which is much more severe than the present outbreak, with a great deal of mortality. I am inclined to doubt that. My reason is that here we had a virus which started to attack people in January in China and which has since affected millions and millions of people all over the globe. It has gone through any number of individuals and has arrived in the United States not any more virulent than it was in Hong Kong back in April or in China back in January. It is hard for me to believe that this virus will suddenly change its character in a few weeks and become extremely severe. That we may have more influenza or a slight rise in influenza in the next few months is possible. They have had it in Japan, another small wave, which is not as big as the wave before. We have had that experience in this country in at least two years. We had a wave of influenza and then a few months later another little wave. I don't think we shall have anything comparable to the severity of the epidemics of 1918, 1919 or 1920.

MODERATOR ROSE: Here is a series of questions, all of which bear on the same topic. Immunization is recommended for "everybody". Would it be advisable to give vaccine to very young infants? At what age would you start? What is the present policy in immunizing children under one year? If a second wave is anticipated, is it advisable to immunize young children?

DR. GREENBERG: At the beginning of the program of immunization when there was only a small amount of vaccine available, many pedia-

tricians who had small amounts on hand gave this to infants of only two or three months of age and to children of older age. We questioned quite a number of these physicians and we found that the reactions in the small infants were very severe. One physician had immunized 30 infants and had 50 per cent reaction. When I say "reaction" I mean that these children had temperatures of 103, 104, 105. There were at least five infants who had convulsions. One of these was the child of a doctor, who had had only 0.05 ml. intradermally. In the older children there were not many reactions.

DR. KILBOURNE: All intradermal injections?

DR. GREENBERG: All intradermal. In view of the fact that the mortality from the disease in the young infant must be very low—as I indicated to you, in our 157 deaths in October we did not have a single one under one year—and the fact that there were very few in children under five years of age, I question the advisability of giving vaccine to young infants. The mortality in the children, even up to 10 and 15, is not high. My own preference would be to give the vaccine primarily to old people, particularly those with chronic heart disease, to pregnant women, to individuals with rheumatic heart disease and perhaps to other adults. I question its value for young children and whether it is worth the risk of giving it to small infants.

The American Academy of Pediatrics has recommended that vaccine be given to infants from three months to five years of age, 0.1 ml. intradermally repeated in two weeks; from 5 years to 13 years of age, 0.5 ml. repeated in two weeks; for older children, 1 ml. in a single dose, subcutaneously.

MODERATOR ROSE: Dr. Hirst, does vaccine confer a greater immunity than an actual attack of influenza?

DR. HIRST: As far as we can judge it must be about the same. The antibody rise one gets with infection and vaccination can be roughly the same. It depends upon how much vaccine is given. One can readily induce a circulating rise of about the same magnitude. This is the only measure we have of the immunity. It may not be the complete answer.

MODERATOR ROSE: What was the cause of the difficulty in distributing the vaccine? I think we must qualify this question. Actually, considering the problems that were encountered in manufacture and distribution, a fairly good job was done.

Here is an interesting question, Dr. Kilbourne: What neurologic

complications are being seen in the present epidemic? What is the prognosis when they occur?

DR. KILBOURNE: Thanks a lot, Mr. Chairman! You know as much or more about this than I do, because I think both of our experiences are derived from the same recent talks in Chicago. We have seen locally—and Dr. Rogers can tell us more about it if he wishes—only one case where we felt that there were any real neurotoxic complications during a syndrome of clinical influenza. It has been a purported problem of the 1918 outbreak which was never established as being solidly related to influenza. There have been associations of various neurologic syndromes with clinical influenza, which apparently have been well documented, that is, the influenza has been well documented. I think all of the cases are as yet inadequately studied with respect to trying to exclude known neurotropic viruses; with so many known neurotropic viruses, that is a formidable problem. With modern techniques it will be of great interest to determine whether neurologic sequelae are really related to influenza.

As Dr. Hirst can tell better than I, there is evidence that some strains of influenza in the laboratory can undergo a mutation to the neurotoxic form. It is conceivable that the virus actually is related to some of these complications.

MODERATOR ROSE: This is along the same line. Dr. Helpern, did any of the patients who died of influenza have evidence of encephalitis?

DR. HELPERN: In one of these cases there were a few petechial hemorrhages in the floor of the fourth ventricle. There were not any diffuse lesions, in the cases we saw, to suggest later development of sequelae in the event of survival.

MODERATOR ROSE: Like the referee at the football game, I shall now inform the team that the two minute mark has been reached. After that period of time this session will be brought to a close. Meanwhile, we shall cover as many questions as we can. There are a number which deal with the use of antibiotics in the management of uncomplicated influenza. I think that Dr. Fisk has covered that fairly well. Here is one question, however, which has to do with the use of antibiotics in the management of uncomplicated influenza in patients with chronic pulmonary disease, and this presents a special problem.

DR. FISK: Because of the fact that individuals with chronic pulmonary disease harbor many bacteria in their lungs which may be activated by an attack of influenza, it is felt that these individuals should be treated

with a broad-spectrum antibiotic to tide them over their period of infection.

MODERATOR ROSE: Dr. Hirst, is there any evidence of antigenic variation among strains of virus during the current epidemic?

DR. HIRST: Not so far. I believe if you will look carefully you will find it, though.

MODERATOR ROSE: We have at least ten questions concerning the efficacy of vaccine given intradermally in a dose of 0.1 ml. to adults. This point has been discussed by Dr. Kilbourne. I believe I said something about it myself. Dr. Kilbourne has evidence that the antibody responses as measured by hemagglutinin inhibition following administration of two doses of 0.1 ml. subcutaneously are equivalent to the responses of 0.5 or 1.0 ml. subcutaneously. However, so far as we can determine from studies in the field, there is evidence that adults get rather better protection from the larger dose of vaccine. This is hardly surprising, because it is a well-known immunologic fact that antibody response is usually related to antigenic mass; also, the character of antigenic response may be related to the periodicity and route of administration of antigen, although the route is generally least important. It is of interest in this connection, as I have already pointed out, that the vaccine has been increased in potency to 400 CCA units per ml., which fact of itself indirectly answers the question about intradermal inoculation.

DR. KILBOURNE: I think it might be re-emphasized that, in my opinion, studies of the intradermal situation itself would suggest that the importance is not the *route* but the *spacing* of multiple injections.

DR. HIRST: It is easy to get some very confusing information on that point. Was your increased response obtained with the spacing of one week with the doses?

DR. KILBOURNE: That is right.

DR. HIRST: I have seen data where inoculation repeated in three to four weeks was without any added response from the second one. Then in a study done by Meikeljohn, where the spacing was six weeks, there was a distinct added effect. So the whole question of double dosage in the absence of any antibody in the population is one which is more or less wide open at the moment and is perhaps more complicated than we might be led to believe from seeing one or two isolated results.

DR. KILBOURNE: I would agree, Dr. Hirst. The only point I was making was that 0.1 ml. given twice subcutaneously, is apparently as good

as the same amount given by the intradermal route. From the limited reports published regarding results of injection via the intradermal route, it would appear that the appropriate control of giving the same dose of virus twice by the subcutaneous route was not followed; where it was followed, indications are that it proved as effective as injection into the skin.

MODERATOR ROSE: I think we have come to the end of our proceedings. I am sorry that we have not been able to answer all the questions which were submitted. For the most part they have been extremely interesting questions and I wish to thank the audience for its participation.

NEW YORK PATHOLOGICAL SOCIETY

ABSTRACTS OF PAPERS AND DISCUSSION

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The Biochemical Cytology of Liver (Abstract)*

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A better understanding of hepatic physiology and pathology will result from application of the techniques of biochemical cytology: electron microscopy, cytochemical staining of cells *in situ*, analysis of subcellular fractions isolated from tissue homogenates by differential centrifugation, autoradiography, and the localization of fluorescent antibody in tissue sections.

In our laboratory we have employed the first three of these techniques in a study of the rat liver cell. The fine structure of this cell, as revealed by electron microscopy, has been summarized elsewhere[1, 2]. Electron microscopy has emphasized the extent to which the structure of mitochondria may be preserved in the process of homogenization and centrifugation[1-3]. The "microsomes" sedimented from this tissue consist largely of fragments of "ergastoplasm" or basophilic material. In the cell, this is a series of inter-connected cytoplasm membranes (endoplasmic reticulum) on the surface of which are arranged the ribonucleoprotein particles of Palade. The endoplasmic reticulum is continuous with the outer of the two nuclear membranes and, at the other end, it may be continuous with the plasma membrane.

From the biochemical study of the isolated fractions has come a vast body of information which emphasizes the complexity and inter-relatedness of the subcellular units[3]. For example, oxidative phosphorylation occurs in the mitochondria. It utilizes DPN**, apparently synthesized in the nucleus, and yields ATP. The latter is utilized in a great many processes, including the initial step in protein synthesis, amino acid activation. This reaction is catalyzed by enzymes recovered in the "supernatant fluid" which remains unsedimented even with high-speed centrifugation Then, in the ribonucleoprotein particles of Palade, the activated amino acids are incorporated into proteins.

There are probably other aspects of dependence of cytoplasmic protein synthesis upon the nucleus. Both chemical evidence and some morphologic observations are consistent with the view that cytoplasmic ribonucleoprotein is derived, at least in part, from nuclear ribonucleoprotein. Nuclear ribonucleoprotein, localized in either nucleolus or chromatin, thus appears to be an important means of transmitting information from genic DNA to the cytoplasm.

Electron microscopy has demonstrated that the plasma membrane surface is in-

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** Abbreviations: DPN, diphosphopyridine nucleotide; ATP, adenosine triphosphate; ATP-ase, adenosine triphosphatase; DNA, desoxyribose nucleic acid; 3'MeDAB, 3'methyl, dimethylaminoazobenzene.

creased enormously in wide areas of the hepatic cell. These are the absorptive surfaces exposed to the sinusoids (space of Disse) and the secretory surfaces exposed to the bile canaliculi[4-6]. It has been possible to localize two important phosphatases in these microvilli by combining electron microscopy with the staining methods of Wachstein and Meisel[7]. Essner and associates[8] showed that the microvilli of the canaliculus surfaces have high levels of ATP-ase and 5'-nucleotidase activities, whereas in those of the sinusoidal surfaces, 5'-nucleotidase, but not ATP-ase, is demonstrable. This enzymatic differentiation of the secretory and absorptive surfaces of the cell probably has important physiological significance. Alterations in such enzymes may be anticipated in disease states. It may be relevant that we find a polarized mitochondrial orientation in the rat liver cell similar to that described by Pollister[9] in *Amphiuma*. Most mitochondria are parallel to a line between sinusoid and canaliculus.

On either side of the bile canaliculi there are cytoplasmic structures the inter-relationships of which still remain to be established. These are the Golgi apparatus, acid-phosphatase-rich granules and "dense bodies".

The multiple nature of the Golgi apparatus is readily revealed by the classical silver or osmium techniques. Careful study shows them always to be situated adjacent to the bile canaliculi[10]. Electron microscopy[4, 6] demonstrates the Golgi bodies of this tissue to have the typical fine structure: parallel membranes and associated vacuoles. (For general reviews of different aspects of the Golgi apparatus see references 11 to 15.)

In 1947 Deane[16] noted that the peribiliary acid phosphatase granules had shapes suggestive of the Golgi apparatus. In work still in progress, we find that under three experimental conditions (bile duct ligation, carbon tetrachloride poisoning, and ingestion of the carcinogen, 3'MeDAB) the Golgi apparatus and acid-phosphatase granules change their intracellular location in a similar manner. However, only with the advent of electron microscopy has resolving power been sufficiently high and the nature of the Golgi apparatus sufficiently defined to permit critical determination of the relation of the acid-phosphatase granules, Golgi apparatus, and other peribiliary structures.

It would be of great importance to establish whether acid phosphatase activity is localized in the peribiliary "dense bodies". In work with Beaufay and de Duve we[17] found such bodies, first described in tissue sections by Rouiller[18], to be present in isolated subcellular fractions which were rich in acid phosphatase activity. It was suggested that perhaps these bodies were the *lysosomes* postulated by de Duve and others[19].

Since the most purified lysosome fractions studied by us contained many mitochondria, it was not possible to establish the peribiliary dense bodies as the sites of acid phosphatase activity[17]. We are currently using electron microscopy in an attempt to localize the enzyme in thin sections of fixed liver incubated in the acid phosphatase medium. In this fashion we hope to clarify the relationship of acid phosphatase granules, dense bodies and Golgi apparatus.

The lysosome concept may be of great importance to pathologists. If de Duve is correct, the hydrolytic enzymes of the cell are contained in small cytoplasmic granules, lysosomes, surrounded by membranes impermeable to the substrates which the enzymes can hydrolyze. Cell autolysis would involve access of substrate and enzyme, through lysosome rupture or more subtle alteration of permeability.

The biochemical cytology of liver must emphasize the quantitative differences among the cells, dependent upon their position in the hepatic lobule. Every cytological structure and enzyme activity which has been studied shows this lobular heterogeneity. There is more ATP-ase and alkaline phosphatase activity in the bile canaliculi of the peripheral zone than in those of the centrolobular zone. On the other hand, 5'-nucleotidase activity is higher in the canaliculi (and peribiliary granules) of the centrolobular cells. The Golgi apparatus is larger and acid phosphatase activity higher in the peripheral cells. Of the microsomal enzymes, glucose-6-phosphatase is very active in the peripheral cells; in the centrolobular

cells it is lower and more variable. Esterase activity is high throughout the lobule, with the highest activity in the centrolobular zone. Lipase and non-specific choline esterase activities are also higher in the centrolobular cells. The mitochondria in the cells of the peripheral zone are long, stout filaments. They become finer as the cells near the central vein are approached. In the latter cells the mitochondria are spherical, fewer in number and very weakly stained in the Baker acid-haematin stain for phosphatide. Correlated with this is the succinic dehydrogenase activity demonstrable by the tetrazolium staining technique; it is very low in the cells adjacent to the central vein and high in the peripheral cells of the lobule. On the other hand, DPNH-Cytochrome C reductase activity as well as the activities of the DPN-linked lactic and beta-hydroxybutyric dehydrogenases, are high throughout the lobule but more so in the centrolobular cells.

It is possible to offer tentative suggestions for the functional significance of some of these quantitative differences. Thus, the area of high glucose-6-phosphatase activity is that in which glycogen is first deposited and first mobilized. The area of highest lipase and esterase activity is that in which lipid first appears, in a variety of experimental conditions. This is also where pigments like lipofuscin are first seen. Finally, the low succinic dehydrogenase activity of the centrolobular cells may be related to the low oxygen tension of the blood entering the central vein. If the visualized succinic dehydrogenase activity reflects the level of aerobic respiration (Krebs cycle) and the visualized lactic dehydrogenase activity the level of glycolysis, this would mean that the centrolobular cells derive a greater proportion of their energy from glycolysis than from aerobic respiration. Such heterogeneity of enzyme distribution may be involved in the selective localization, in the hepatic lobule, of lesions due to poisons, toxins and infectious agents.

The cell changes familiar to pathologists are beginning to be re-examined with the newer techniques under discussion. Thus, cloudy swelling in a variety of circumstances appears to involve a rounding and enlargement of the mitochondria in which the inner membranes undergo profound change[20].

Recently we studied a liver biopsy from a 72 year old man, made available to us by Dr. Joseph Ehrlich. The fine structures of nucleus, mitochondria, ergastoplasm and bile canaliculi are essentially similar in human and rat liver. The same is true for the staining of the bile canaliculi for ATP-ase, 5'-nucleotidase, and alkaline phosphatase; of the peribiliary granules for acid phosphatase; and of the mitochondria for succinic dehydrogenase, DNP-linked and TPN-linked dehydrogenases and associated cytochrome C reductases. We shall mention but one difference, to indicate that species differences do exist and to suggest its possible clinicopathologic interest. In human liver, unlike that of the rat, there is intense alkaline phosphatase activity on the sinusoidal aspects of the cells. Indeed, there is much more activity there than in the bile canaliculi, and perhaps more attention should be paid to it in human liver disease.

By studying cells with varying amount of lipofuscin we have tentatively arrived at a description of the changes which may occur in the hepatic cell. The first lipofuscin granules are formed adjacent to the bile canaliculi; their appearance coincides with the disappearance of the peribiliary acid phosphatase granules. It is of interest that Pfuhl[21] suggested, on the basis of the peribiliary location of the granules, that lipofuscin appears within the Golgi bodies.

As lipofuscin droplets become more frequent they are also found further from the canaliculi. When the droplets become very numerous, it seems that the ATP-ase and 5'-nucleotidase activities disappear from the canaliculi and the canaliculi themselves can no longer be seen. There is, however, no diminution in the activities of the dehydrogenases studied (succinic, lactic, beta-hydroxybutyric) and of DPNH-cytochrome C reductases, until perhaps the very end in some cells which may be dying. Nor is there any apparent diminution of sinusoidal enzyme activity.

In electron micrographs (of tissue fixed in osmium tetroxide) the lipofuscin granules consist of a homogeneous electron-dense material arranged around irregular

vacuoles. Few, or even single, vacuoles are noted in bodies about the size of mitochondria, or somewhat larger. In the large lipofuscin droplets there are a great many vacuoles of various shapes. Large spherical droplets, presumably lipid, are frequently found in the same cells. These have thin irregular electron-dense coating, and a small inner granular core. The largest part appears empty, suggesting extraction of material during processing of the tissue. Perhaps the outer layer corresponds to lipofuscin.

Further studies, with animals on experimental regimes as well as with human material, will be required to establish any relations that may exist between lipofuscin granules and either mitochondria or the Golgi apparatus.

It has been our purpose to convey a general picture of the biochemical cytology of the liver and to suggest the value of its techniques to problems of pathology and aging.

Mechanism of Hepatic Fibrosis

(Abstract)

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The important aspects of hepatic fibrosis are its types, histogenesis, histochemical and chemical nature, and its functional significance. These were studied in human biopsy and autopsy material and in animals experimentally intoxicated with ethionine, carbon tetrachloride or thioacetamide or placed on low protein/high fat diets.

Fibrosis may be solely in the portal tract, in the periportal or centrolobular zones, anywhere within the lobule, and focal. These types of fibrosis do not disturb the lobular architecture in contrast to cirrhosis characterized by regenerative nodules and connective tissue septa. The pathways by which cirrhosis develops are: 1) collapse following disappearance of liver cells (postnecrotic cirrhosis), 2) septa formation most often associated with fatty metamorphosis but also with chronic passive congestion, granulomas and hemochromatosis (septal cirrhosis), and 3) inflammation and scarring around bile ducts and ductules either primary or secondary to extrahepatic biliary obstruction (biliary cirrhosis). The three basic pathways do not each imply a specific etiology and are associated with several types of fiber formation.

Basic observations in other organs apply to collapse and fiber formation in the presence of fibroblasts in the liver. A particular problem is fiber formation around fatty or damaged liver cells and around ductular cells. For this it was advantageous to reexamine the distribution of fibers in the normal liver, particularly in sections one micron thick (studies with F. Paronetto and H. van der Noen).

The border between the normal liver cell and the sinusoid is marked by an amorphous and partly fibrillar layer which gives the periodic acid Schiff reaction after removal of glycogen by diastase (DPAS) and a few small DPAS positive granules are in the Kupffer cells. Chromotrope aniline blue (CAB) stains the border zone hazy purple with fine blue reinforcements which can be impregnated with silver. This reticulum appears in thin sections as a row of points or dashes indicating cross or tangentially cut fibers. Around ductules (cholangioles) a continuous black line is seen in silver stains

indicating a basement membrane which also gives DPAS reaction and is blue in CAB stain.

Around damaged liver cells in hepatitis or cirrhosis, a thicker amorphous layer is purple in CAB stains. Frequently a few irregular DPAS positive granules are found in the liver cells and denser granules which vary greatly in size are in the Kupffer cells. On the base of the liver cells a continuous DPAS positive line appears while in silver impregnations a dense row of points or dashes suggests increase in reticulum fibers or formation of a basement membrane similar to that of ductular cells. Around fatty liver cells such a structure is also noted which is sometimes even duplicated and in extreme cases gives a collagen reaction. Fibroblasts are not conspicuous. In rats with ethionine intoxication producing single cell necrosis and hepatocellular degeneration, DPAS positive granules in liver cells and Kupffer cells and the amorphous and fibrillar material on the base of the liver cell are very conspicuous. In addition, cells with oval- or spindle-shaped nuclei accumulate between the liver cell plates (oval cells of Farber). They are occasionally arranged as ductules and a fine canalicular system between these cells can be visualized by injecting the bile ducts with India ink. This indicates their epithelial origin despite their frequently mesenchymal appearance. Around such ductular cells, much DPAS-positive amorphous and fibrillar material can be seen. Silver impregnates fibers or membranes which may be multilayered even in thin sections. Some of the fibers and membranes in contact with the ductular cells continue away from them into the surrounding tissue. Fibroblasts are again not demonstrable. This relation of fiber formation to ductular cells is also noted in man, e.g., in cirrhosis and in biliary obstruction.

The fibrillar material, impregnated by silver with a positive DPAS and blue CAB reaction, becomes metachromatic after sulfation and is not digested by trypsin or hyaluronidase. It is therefore reticulin and transitions into collagen which are observed in silver impregnation and van Gieson stains. The amorphous substance on the sinusoidal border of the liver cells and the granules in the damaged liver cells and proliferating Kupffer cells which give a DPAS reaction and are purple with CAB become also metachromatic on sulfation and partially react with colloidal iron and Alcian blue. The material is digested by trypsin but not by hyaluronidase, pectinase or ribonuclease, and it loses its PAS reaction on acetylation. It is apparently a polysaccharide protein complex which may be a matrix for the formation of scleroprotein fibers, collagen and reticulum. The question is left open as to whether the similarity in the histochemical reaction of the perisinusoidal material and the granules in the Kupffer cells indicates that the latter engulf the former, acting as scavenger cells, or whether the cells form it and thus assume the function of fibroblasts. The formation of fibers in this matrix around the damaged liver cells and ductular cells suggests that these structures play an inducing role in determining the site and extent of the fibrosis. Since the ductular cells are possibly derived from liver cells, dedifferentiation may be the common factor in stimulating the fibrosis.

Further support is obtained in recent experiments (carried out by F. G. Zak, F. Paronetto and I. Bubelis) with carrageenin, a polysaccharide derived from moss. Within two days after its injection into the liver, a necrotic area is surrounded by a ground substance giving acid mucopolysaccharide reaction in which fibroblasts seem to develop from adventitial cells of arteries and capillaries and from Kupffer cells. The ductular cells also proliferate profusely and sometimes ductular cells cannot be distinguished from fibroblasts. Abundant reticulum is formed particularly in approximation to the ductular cells although some fibers continue away from them. The violent fibroblastic reaction which completely subsides in two weeks illustrates the rapid formation of ground substance and reticulum in the vicinity of fibroblasts and ductular cells.

To supplement the histochemical studies, the amino acid, hydroxyproline, which occurs almost exclusively in collagen, was determined in dried and defatted liver tissue and in a fraction extracted by 0.1 N NaOH believed to contain procollagen (in-

vestigations of F. Hutterer and E. J. Singer in human cirrhosis). The increase of collagen is associated with a relatively greater increase of the alkaline soluble fraction. Serial experiments indicated that the hepatic hydroxyproline and hexosamine content rises precipitously to three times the normal during the seven weeks on an ethionine diet. Much of this is in the NaOH soluble fraction and coincides with a marked increase of the reticulum histologically. A rise in proline in the alkaline soluble fraction only precedes the rise in hydroxyproline, suggesting a precursor relationship. Therefore, during active fibrosis, polysaccharide appears around liver cells while procollagen and then collagen is formed, utilizing hepatic proline which is converted to hydroxyproline.

In assessing the functional significance of hepatic fibrosis, the interposition of a mucoprotein or scleroprotein material between sinusoids and liver cells is thought to interfere with some hepatic functions such as the free exchange of metabolites between the liver and blood. The interruption of bile flow through such a connective tissue accumulation is demonstrated in primary or secondary biliary cirrhosis in which the obstructive features parallel the development of a thick fibrotic layer. The connective tissue septa in cirrhosis carry anastomotic vessels between the hepatic artery, portal vein and hepatic veins, shunting blood from the hepatic parenchyma and transmitting arterial pressure into the portal system, thereby creating many of the cardinal symptoms of cirrhosis.

In conclusion, hepatic fibrosis poses the same problems as accumulation of fibrous tissue elsewhere, modified by the determining role of altered epithelial elements and by the consequences that are related to the functions of the liver and its double blood supply.

DISCUSSION

MAX WACHSTEIN: I really think that this is a memorable meeting, since it must bring to everyone's mind that pathology is now in a period of dynamic transformation. It is about 100 years since Virchow introduced the concept of cellular pathology which has remained the basic foundation of our discipline. During the last decades, the impact of this concept has lost much of its scientific drive. However, within the last years, new techniques have been developed to such a degree that they now can be used for the study of normal and diseased tissues. Just to name a few: spectrophotometry, fluorescent microscopy, histochemistry, phase microscopy, electron microscopy and others. With the aid of these new techniques, cellular pathology can now be explored in terms of functional pathology and the study of cells can be extended to subcellular and even molecular levels.

It is impossible to discuss the wealth of material presented today. Just to comment briefly on one point made by Dr. Novikoff: dehydrogenases were first determined in tissue extracts by biochemists. The great improvement in histochemical techniques both with regard to fixation as well as to the reagents used enables one to localize specific dehydrogenase, as Dr. Novikoff has shown, in various portions of the liver lobules; DPN mediated dehydrogenases, for instance, are found mainly around central and succinic dehydrogenase around periportal fields. In addition to the topographical localization, these stains demonstrate the mitochondria as sites of enzymatic activity. Most recently Pearse and Scarpelli[22] have even been able to identify specific areas within isolated mitochondria as sites of dehydrogenase activity. It seems perfectly feasible that changes in enzymatic components of mitochondria may characterize various disease states which cannot be distinguished at present with the usual routine techniques. To take another example: if one considers the difficulty of proper diagnosis of the various forms of liver disease, one cannot help but be hopeful that the application of the electron microscope, with or without various enzyme staining techniques, will become very helpful in their understanding and differentiation. Cholestasis, for instance, occurs in many abnormal conditions. It seems obvious that the study of the bile canaliculi with the electron microscope, aided possibly by the adenosine tri-

phosphate technique, will be of help in the understanding of the cause for this abnormality.

As regards Dr. Popper's presentation, I think it is very important that he stressed the fact that fibrosis in the liver does not always originate in the fibroblasts. It is difficult to comment, in a short discussion, on the variety of histologic and biochemical techniques he has used. I would, however, like to make the following specific point: in the rats given ethionine—he has not mentioned that he, himself, first described the interesting ethionine changes in the liver—we have consistently found fairly extensive areas of central necrosis which he also mentioned in one of his papers[25]. These central areas of necrosis later tend to extend into periportal fields and it is sometimes difficult to say from where they started. Similar areas of atrophy and necrosis have been described by Opie in rats fed butter yellow[24]. In these areas, fibroblastic proliferation was described and has also been shown by Morrione[23] to occur in animals treated by repeated inhalations of carbon tetrachloride or fed butter yellow. It would thus appear that the total amount of hydroxyproline measured chemically may also be derived from collagen produced by typical fibroblasts.

I thought I might contribute most to this discussion by showing a few pertinent slides. In a section of a normal human liver biopsy, the bile canaliculi stained very well with the adenosine triphosphatase technique[7]. When liver biopsies of cases of jaundice were studied, one could see how the bile canaliculi extended toward the sinusoids thus making it perfectly understandable that in biliary obstruction reflux from the bile into the liver may occur[26]. In this connection, I would like to ask Dr. Novikoff whether in his electron micrographs, the Disse spaces can be made out as consistent structures, which separate the bile canaliculi from the sinusoids, as has been suggested by Rouiller[6].

Enzymatic staining reactions in ethionine-induced liver damage reveal interesting changes. The ductular cells which Dr. Popper has discussed show a very marked depression of enzymatic activity as compared to normal liver cells. In contrast, regenerating liver lobules show a very strong reaction for various enzymes. If ethionine administration is continued long enough, hepatomas will occur. In such hepatomas alkaline phosphatase activity showed a very strong reaction[27].

In concluding this discussion, I should like to say that all of us who are practicing hospital pathologists are most grateful for these presentations which so clearly reveal the areas into which scientific pathology is now moving. It is quite obvious that hospital pathologists cannot do all of this type of work; we do not have the equipment and often not the time, but nevertheless hospital pathologists like myself can work in any of the new fields that have been opened by these new techniques. The possibilities are endless and the reward is the satisfaction of participating in this exciting phase of scientific pathology.

HANS POPPER: The presence of fibroblasts in the rat liver after ethionine intoxication is a problem that has puzzled us for a considerable time. Variation of ethionine diets produces a great variety of hepatic injuries and, while some procedures produce unquestionable central necrosis, the animals of the series studied chemically in our laboratory for developing fibrosis failed to reveal such necrosis. In these specimens no typical fibroblasts were found. However, I would like to emphasize that Dr. MacDonald of the Boston City Hospital has seen beautiful fibroblasts in rat cirrhosis induced by low choline/high fat/low protein diets, and we have seen them in some instances of ethionine cirrhosis. In the latter case, the question arose as to whether these were actually fibroblasts or modified ductular cells, particularly since we could inject between these cells channels from the biliary system. This problem is also illuminated by transitions between ductular cells and fibroblasts seen particularly well in carrageenin granulomas within livers of rats treated with ethionine. I am therefore not able to answer your question because I am not quite sure any more what a fibroblast really is and how to differentiate it occasionally from cells of apparently epithelial origin.

ALEX B. NOVIKOFF: In the present stage of development of electron microscopy it would be foolhardy to suppose that "seeing is believing". We would not categorically assert that artefacts are not involved in the many discontinuities seen in the cells lining the sinusoids or the spaces seen between the lining cells and the hepatic cell surfaces. However, it seems unlikely that shrinkage or other artefacts would produce such well-structured microvilli as are found on the cell surfaces in that region. These are like the microvilli found in many tissues where cells are exposed to cavities. It seems reasonable to suppose: 1) that the lining cells do not completely separate the liver cells from the sinusoids, and 2) that the microvilli project into a plasma-filled space, the Disse space. Just as the excretory surface at the bile canaliculus is increased by microvilli so is the absorptive surface at the Disse space. The two surfaces are probably differentiated enzymatically, as our findings with phosphatases indicate.

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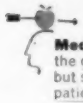

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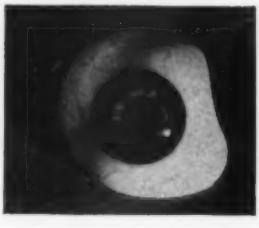

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"The paradox of migraine – increased nausea due to ergotamine administration – may now be successfully combated with 'Migral'. The recognized benefits of ergotamine and caffeine in 'Migral' are favorably enhanced by the addition of cyclizine hydrochloride, a specific to overcome nausea.

Dosage: 2 to 3 tablets at first warning of an attack, then 1 or 2 tablets every half hour; not more than 6 tablets should be taken for any single attack.

Supplied: 'Migral' tablets, containing ergotamine tartrate 1 mg., 'Marezine'® brand Cyclizine Hydrochloride 25 mg., and caffeine 50 mg.

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